ALLELOPATHIC EFFECTS IN ASSOCIATION OF WHEAT SOIL AND STRAW WITH DETERMINATION OF SELECTED ALLELOCHEMICAL STRUCTURES

BY

RUOWEI LORI ZHU THORNE

Bachelor of Science in Chemistry

Beijing University

Beijing, China

1984

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1987

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Thesis approved:

George R. Waller
Thesis Adviser

Andrew J Mort

Margaret K. Essenberg

Norman N. Durham
Dean of the Graduate College

ACKNOWLEDGMENT

I wish to express my greatest appreciation to my major advisor Dr. George R. Waller for his advice and guidance throughout this study. My gratitude goes to my advisory committee: Dr. James K. McPherson for his instruction and advice during the research; Dr. Margaret K. Essenberg for her suggestions on course work, which were so crucial for the completion of this study.

Very special thanks are expressed to my husband Barry for his moral support, encouragement, confidence in my abilities and sincere appreciation of this study. My thanks are likewise extended to my parents, who gave me emotional help all the way through from far-away China; and my parents-in-law, who showed their great concern with my research.

To Dr. Otis C. Dermer who proof-read this thesis and gave expert comments I extend my heartfelt thanks. I appreciate Dr. Andrew Mort substituting for Dr. McPherson while he was in Australia on a sabbatical-leave. My project colleagues Kevin Cast and Cindy Wyman deserve recognition for their cooperation and enlightening discussions. Gratitude is extended to the Department of Biochemistry for awarding me the research assistantship and to many staff members in the department for their help.

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LIST OF ABBREVIATIONS

aq.	-	aqueous
bu/acre	-	bushel(s) per acre
^{o}C	-	degree centigrade
ca	-	about
CGC/MS/DA	-	gas chromatography/mass spectrometer/ data analysis
CH_2Cl_2	-	methylene chloride
$CHCl_3$	-	chloroform
CH_3OH	-	methanol
cm	-	centimeter
conc.	-	concentration(s)
CT	-	conventional-tillage
DNA	-	deoxyribonucleic acid
ev	-	electron volts
ext.	-	extraction
fig.	-	figure(s)
fract.	-	fraction(s)
g	-	gram
g/m^2	-	grams per square meter
h	-	hour(s)
H_2O	-	water
inhib.	-	inhibition
KV	-	kilovolt(s)
lb./acre	-	pounds per acre

LIST OF ABBREVIATIONS (Continued)

lyoph. - lyophilization; lyophilized

m - meter

M - molar

min - minute(s)

ml - milliliter

mm - millimeter

MS - mass spectrometry

M.W. - molecular weight

N_2 - nitrogen

NAOH - sodium hydroxide

nm - nanometer

No. - number

NT - no-tillage

p. - page(s)

ppm - parts per million

rpm - rotations per minute

stimu. - stimulation

µa - microampare

µm - micrometer

CHAPTER I

INTRODUCTION

The term allelopathy was coined by Molisch in 1937 to refer to the inhibitory or stimulatory effects of one species on the growth and development of another among plants of all levels of complexity, including microorganisms (1). Allelopathically active biochemicals are called allelochemicals, and most of them are secondary metabolite produced as by-products in plant primary metabolic processes.

Allelopathy is more evident in agronomic systems than in natural ones because there has been little opportunity for either spatial or evolutionary adjustments in agronomic settings. In natural communities, allelopathy is apparently fairly common, but is less apparent because of such adjustments (2, 3). It has frequently been observed that stubble mulch or no-tillage farming of wheat (Triticum aestivum), i.e, leaving wheat straw on the surface of soil, reduced the growth and yield of following wheat crops compared to those crops with which conventional tillage was used (4, 5, 6, 7, 8). It is important, therefore, to investigate the soil organic substances that are either directly released from decomposing residues or soil microbe metabolites that affect the crop development and production.

Accumulated evidence prompted this study on the isolation and identification of allelopathic substances that cause this particular phenomenon. Wheat straw and soil samples of both conventional-tillage and no-tillage plots were chosen as experiment materials. Techniques and procedures were developed to isolate the naturally occurring phytotoxins. The objectives of the research were: (a) to extract and isolate the allelopathic agents from wheat straw residues and field soil under the mildest conditions in order to conform most closely to the natural situation; (b) to bioassay the potentially allelopathic extracts and sequential fractions by testing their biological activities on germination and early growth of wheat; and (c) to characterize chemically the compounds found in the extracts that are allelopathic by using a combined capillary gas chromatography/mass spectrometry/data analysis technique. Qualitative studies on the identification of these phytotoxins was partially determined. A chemical linkage between source and influence of the allelochemicals in the association of wheat straw and soil system was established.

CHAPTER II

LITERATURE REVIEW

A. Introduction of Allelopathy

To trace the history of allelopathy, Theophrastus (9) was probably the first person, at about 300 B.C., to record the phenomenon presently termed allelopathy. He observed that chick pea (Cicer arietinum) does not reinvigorate the ground as other related plants (legumes) do, but "exhausts" it instead. Similar phenomena were later reported by Pliny (10). He maintained that chick pea, barley (Hordeum vulgare), fenugreek (Trigonella foenum-graecum), and bitter vetch (Vicia ervilia) all "scorch up" cornland. As knowledge of ecological phenomena advanced, De Candolle (11) suggested that the soil sickness problem in agriculture might be due to exudates of crop plants and that rotation of crops could help alleviate the problem. In spite of frequent observations and descriptions of concerning allelopathic phenomena, no controlled scientific research was conducted until the nineteenth century (12).

Allelopathy differs in mechanism from other plant interference, such as competition. Competition denotes the differential potential of organisms to gain access to environmental resources, such as light, space, or water,

while allelopathy functions through the releasing and concentrating of chemicals in the immediate environment. Evidence indicates that the release of allelopathic chemicals into the environment may proceed through: volatilization from plant tissues, exudation from roots, leaching from plants or residues by rainfall, and decomposition of residues (12).

The most important properties of allelopathic events are: (a) allelochemicals produced by one plant species may affect either the growth of other plants (phytotoxin) or itself (autotoxin); (b) allelochemicals may not only be growth inhibitors or stimulants, but these effects may also be interchangeable; allelopathic inhibitors may become stimulants at very low concentrations, about 10^{-4} to 10^{-6}M in most cases, and (c) many allelochemicals, when below a threshold concentration, often function in an additive or/and synergistic way rather than individually (12).

A wide range of microbial activities are involved in allelopathic phenomena. Toxins may be produced by soil microbes during their metabolism or during microbial decomposition of specific plant residues (12).

Studies of allelopathy have increased during the last decade owing to the increasing realization among agricultural scientists, plant ecologists, and plant physiologists that it influences the presence and growth of plants and the nature of plant communities under many circumstances. The incorporation of allelopathic effects for practical agricultural ends appears likely in the near

future. One probable application is to use natural allelochemicals as starting materials for synthesizing herbicides, pesticides and fungicides, thus avoiding petroleum-based compounds, which have caused serious pollution and carcinogenic problems.

B. Research Done by Others

Current statistics showed a steady increase in conservation tillage (no-tillage) practices (1.5 % increase per year) across the United States (about 15 % of the crop acres in 1974 to over 30 % in 1984). Besides the benefits in reducing farming costs, preventing soil erosion and maintaining soil moisture in conservation tillage planting may be the most attractive aspects for its adoption. The increase in no-tillage farming in Oklahoma is great (a 500,000-acre increase in 1984 over 1983). Studies done by Stiegler and Krenzer on wheat farming during 1983-1984 (13) indicated that no-tillage systems allowed early planting and better early growth of wheat, and hence better forage yields, compared to conventional-tillage planting. The average forage yield for no-tillage plots was 3,000 lbs/acre for August-planted wheat, and there was no germination at all in comparable moldboard plow plots. The reason for this phenomenon was thought partially as the crop residues on the surface of the soil in no-tillage plots significantly lowered the soil temperature and kept the soil moisture from evaporating. Therefore a more favorable environment was established for wheat germination and early growth. This

disadvantage of moldboard plow plots in early planting was reversed , however, in later plantings (wheat planted in late fall and winter) owing to the higher soil temperature in moldboard plow plots than in no-tillage plots during the growing period. The warmer soil, as the authors considered, helped the wheat plants to grow more and consequently contributed to the higher production (wheat yield was 51 bushels per acre for moldboard plow plots and 38 bushels per acre for no-till plots). These comparison indicated that both conventional-tillage planting and no-tillage planting are adoptable if they are practiced at different seasons properly with consideration of soil temperature and soil moisture.

Although the national trend is toward increased use of conservation tillage systems, the lack of substantial production increases has prevented no-tillage practices in wheat farming from being widely adopted. Obviously other biological factors such as weed control, diseases, insect damage, and allelopathy need to be considered. These considerations may be more critical for wheat production than soil temperature because it has been observed that yields of forage and grain in no-tillage and conventional-tillage wheat varied also with other factors, such as rainfall and geographic locations (13). Among those other factors allelopathy is one of the most active research subjects. The research on allelopathy aims to demonstrate the effects of crop residues on wheat growth.

Ecologists and agricultural scientists have been

studying the causes of allelopathic phenomena in crop production for many years (14, 15). The phytotoxicity associated with crop residues is a widely noticed problem with no-tillage systems. McCalla and Army (4) observed that yields declined when stubble-mulch tillage was practiced. They also postulated that the yield reduction was residue-related since the wheat yield decreased with increasing annual precipitation. Further studies indicated that this phenomenon was not related to nitrogen deficiency, as the reduction was not corrected by nitrogen applications (7, 6, 16). This suppressing effect of crop residue on the productivity of successive crops has prompted many studies on the various possible factors involved under actual field conditions. Obviously the soil environment responds differently to crop residues on the soil surface than to residues buried by plowing.

McCalla *et al*. (17) found less residue decomposition and higher quantities of total nitrogen, organic matter, and microorganisms in the surface 2.5 cm of soil with stubble-mulch (remains of wheat plants after harvest allowed to remain *in situ*) than with plow tillage. A later report in Oklahoma (6) gave a similar observation on organic matter content in mulched wheat plots from the soil surface down to 30 cm. The research done by Iswaran and Harris (18) indicated that anoxic conditions in soil may be greatly promoted when large amounts of residues are present, and that phytotoxic agents could be extracted from the decomposing residue.

Some microbes incorporate compounds released from decomposing plant residues as substrates into their metabolism. They may then produce allelochemicals that can either positively or negatively influence plant growth (12, 19, 20, 21). Accumulated residue on the surface of the soil also tends to hold more moisture, and this promotes microbial activities (4, 22, 23).

Tang and Waiss (24) isolated several short-chain fatty acids, such as acetic acid, propionic acid, and butyric acid. These are common microbial fermentation products in anaerobic metabolic conditions and they were shown to be phytotoxic in wheat seedling growth tests. Similar results were also reported by Lynch (25) and Prill (26).

In Australia, Kimber (7) noted that wheat planted in no-tillage plots after the first rains that followed the dry weather produced the poorest yields. In further phytotoxin studies from decomposing plant residues, he obtained a maximum amount of water-extractable phytotoxins from wheat straw after 2 to 6 days of decomposition, while no water-extractable phytotoxins were obtained after 54 days of decomposition. The toxins extracted affected the growth of early seedlings of wheat, with more inhibition of root growth than of shoot growth (27, 28). Kimber found that extracts from partially decomposed cereal and legume residues were more toxic than those from dried straws. This suggested that toxin production was cyclic and seasonal.

Plants produce as well as respond to toxins differently. Cochran et al. (5) studied the phytotoxin

production of a series of plant straws layered on the soil surface. The residues of wheat, barley (Hordeum vulgare), pea, lentil, and bluegrass (Poa pratenis), were collected weekly through August to the next May. No water-extractable toxins were found until after rainfall in late September. Pea and lentil residue extracts showed as much as 90% inhibition on root elongation at the beginning, but the toxin production decreased rapidly, and disappeared during winter and spring. Wheat and barley residues intermittently produced phytotoxins throughout the fall, winter, and spring. Bluegrass straw did not produce phytotoxins until late in the fall and these reached a maximum of 60% inhibition in the spring.

Waller et al. (9) collected Oklahoma wheat soil from April through July, 1985. They treated the soil with various organic solvents and steam distillation, and bioassayed the extracts on seedling growth of wheat. Their results indicated that both conventional-tillage soil and no-tillage soils contained inhibitory substances, some of which showed very toxic effects on early wheat growth.

Recently, progress has been attained in identification of allelochemicals in weeds and crops, and in investigating the modes or mechanisms through which these allelopathic agents function (30, 31, 32). Lovett and Levitt (32) concluded that allelochemicals were involved in plant defense systems to protect against other organisms and to give themselves advantages over such organisms. This suggested to them that allelopathy is enhanced by natural

selection.

Several groups of chemicals isolated from fresh and decomposing crop residues have been reported as allelopathic agents (33). Among these, phenols and phenolic acids are the most frequently identified. Ferulic acid, p-coumaric acid, vanillic acid, and p-hydroxybenzoic acid are representatives of those allelopathic phenolic acids (22). Some short-chain fatty acids, which are anaerobic metabolites as already mentioned, are inhibitory to root elongation (24), whereas the products of the aerobic process stimulated the extension of barley seedlings (25). Some aerobic bacteria and fungi produce antibiotics that may also be toxic to plants. One of those is patulin ($C_7H_8O_4$; see structure in Table I), an antibiotic produced by Penicillium urticae Bainer (34). Other common types of allelochemicals are unsaturated lactones, long-chain fatty acids, straight-chain alcohols, aliphatic aldehydes, ketones, complex quinones, terpenoids, steroids, flavonoids, cyanohydrins, and alkaloids (12, 22, 23, 34, 35). Some allelochemicals isolated from plant residues and soil are listed in Table I and Table II.

Although a great number of allelochemicals have been isolated from plant tissues and soil environment in fields, greenhouses, and laboratories, many were extracted under somewhat unnatural conditions rather than by collecting the responsible extracellular toxic compounds released in the natural environment. This is probably due to the difficulties of sampling from intact, living plants. Thus

TABLE I

REPRESENTATIVE ALLELOPATHIC COMPOUNDS
ISOLATED FROM PLANT RESIDUES

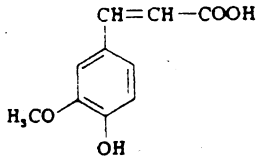
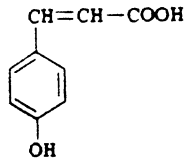
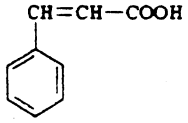
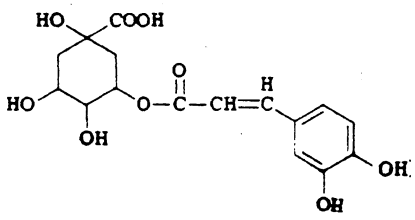
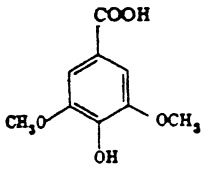
Chemical Class	Name	Structural Formula	Reference Cited
Phenolic acids	Ferulic acid		12, 22
	p-Coumaric acid		
	Cinnamic acid		
	Chlorogenic acid		
	Syringic acid		

TABLE I (Continued)

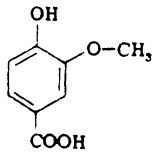
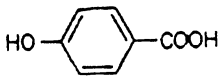
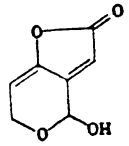
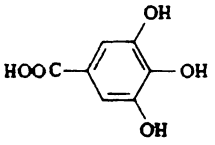
Chemical Class	Name	Structural Formula	Reference Cited
Phenolic acids	Vanillic acid		12, 22
	p-Hydroxybenzoic acid		
Simple lactone	Patulin		34
Tannin	Gallic acid		12
Long-chain fatty acids	Myristic acid	$CH_3(CH_2)_{12}COOH$	37, 38
	Palmitic acid	$CH_3(CH_2)_{14}COOH$	
	Stearic acid	$CH_3(CH_2)_{16}COOH$	
	Oleic acid	$CH_3(CH_2)_7C{=}C(CH_2)_7COOH$ (H H)	

TABLE I (Continued)

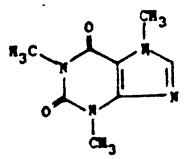
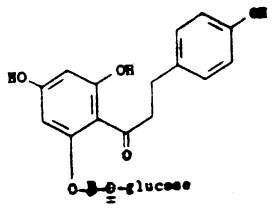
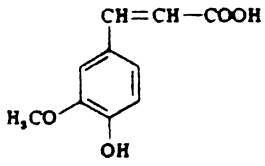
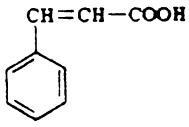
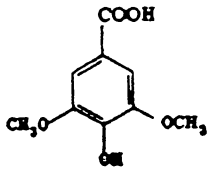
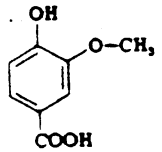
Chemical Class	Name	Structural Formula	Reference Cited
Long-chain fatty acids	Arachidic acid	$CH_3(CH_2)_{18}COOH$	37, 38
	Behenic acid	$CH_3(CH_2)_{20}COOH$	
Short-chain fatty acids	Acetic acid	CH_3COOH	24
	Propionic acid	$CH_3CH2COOH$	
	Butyric acid	$CH_3CH_2CH_2COOH$	
Alkaloid	Caffeine		39
Flavonoid	Phloridzin		12, 40

TABLE II

REPRESENTATIVE ALLELOPATHIC COMPOUNDS ISOLATED FROM SOIL

Chemical Class	Name	Structural Formula	Reference Cited
Short-chain fatty acids	Acetic acid	CH_3COOH	24, 25
	Propionic acid	CH_3CH_2COOH	
	Butyric acid	$CH_3CH_2CH_2COOH$	
	Pentanic acid	$CH_3CH_2CH_2CH_2COOH$	
Long-chain fatty acids	Palmitic acid	$CH_3(CH_2)_{14}COOH$	12, 37
	Stearic acid	$CH_3(CH_2)_{16}COOH$	
	Arachidic acid	$CH_3(CH_2)_{18}COOH$	

TABLE II (Continued)

Chemical Class	Name	Structural Formula	Reference Cited
Long-chain fatty acids	Oleic acid	$CH_3(CH_2)_7\overset{H}{C}=\overset{H}{C}(CH_2)_7COOH$	12, 37
	Linoleic acid	$CH_3(CH_2)_4\overset{H}{C}=\overset{H}{C}CH_2\overset{H}{C}=\overset{H}{C}(CH_2)_7COOH$	
Phenolic acids	Ferulic acid		7, 35
	Cinnamic acid		
	Syringic acid		
	Vanillic acid		

compounds that have been extracted and identified as allelochemicals in the laboratory may not be responsible for the observed allelopathic effects in nature. Tang and Young (36) developed a simple but excellent system for continuously trapping root exudates that allowed recovery of toxic exudates from undisturbed bigalta limpograss (Hemarthria altissima) root systems. The chemicals were adsorbed on a XAD-4 polystyrene resin (Rohm and Haas), through which a nutrient solution was circulated. Extracellular hydrophobic metabolites were selectively retained by the resin, while inorganic nutrients were recycled to maintain the plant growth. The chemicals were then eluted from the resin for characterization. Twelve compounds extracted in this manner were identified using gas chromatography/mass spectrometry/data analysis (GC/MS/DA). Among them several phenolic acids and some of their derivatives are known allelochemicals (36).

Within a complex soil system naturally phytotoxic compounds may exert their effects when sufficient concentrations are accumulated by adsorption or when their functions are controlled enzymatically. Suflite and Bollag (41) found that a soil-enzyme complex could oxidize some phenols, such as resorcinol, 1-naphthol, and 4-chloro-1-naphthol, to polymers. These polymers were assimilated as a part of the soil matrix and would not be released until later as allelochemicals.

The entire subject of allelopathy was reviewed by Rice (12) and his book has been responsible for more agricultural

scientists becoming interested in this study. Waller (42) provided a broad coverage of current allelopathy research in agriculture and forestry. Thompson (43) and Putnam (44) also reviewed international research on this subject. Recently many scientists have attempted to apply the results of the study in agricultural practices, e.g., in weed control, residue management, crop production improvement and genetic engineering (plant DNA recombination).

CHAPTER III

MATERIALS AND METHODS

A. Sample Materials

1. Location of the fields

Wheat soil and straw samples were collected from plots located in the Agronomy Farm, Efaw Plots, Stillwater, Oklahoma. Sorghum was planted prior to 1982, and then followed by continuous wheat plantings until the present time. These plots are part of an on-going research project that measures the influence of soil and residue management systems on wheat production. This multidisciplinary research project is conducted by the Oklahoma Agricultural Experiment Station.

2. Characteristics of the samples

The soil type from which all soil and straw samples were taken is a Pulaski coarse, loamy, mixed thermic Typic Ustifluvent (fine sandy loam with 0-2 percent slope). Mean annual precipitation is 82 cm (Climatological Data, Agronomy Department, Oklahoma State University, Oklahoma).

3. Treatment of the soil

a) Conventional tillage -- residues were turned under

as thoroughly as possible with a moldboard plow which inverts and mixes the soil about 20-30 cm deep.

b) No tillage -- all residues were left on the surface of the soil. These plots have been maintained as no-tillage plots since 1981.

4. Sampling of the soil

Representative soil samples (down to 5 inches deep) were taken at harvest (10 June, 1985) by subsampling (4-6 times) of both the conventional-tillage and the no-tillage plots. The sub-samples were combined, and thoroughly mixed before being placed in quart Mason jars, and immediately frozen with dry ice in the field. They were then transported to the laboratory and stored in the freezer at -18 ^{0}C until further use.

5. Sampling of the wheat straw

Two types of wheat straw were collected:

a) new wheat straw -- newly dropped by the combine.

b) old wheat straw -- left on the surface of the soil after combine harvest in the previous about three years.

Samples of wheat straw were taken at harvest (10 June,1985) in the no-tillage plot only and air-dried. The air-dried straw was ground to pass through a 20-mesh screen (U.S. standard) by using a Micro Wiley mill, and stored at room temperature.

B. Procedure

1. Introduction

Owing to the low concentration of biologically active substances and the complexity of soil systems with their various soil enzyme and microbiological activities, extraction and fractionation of allelochemicals from a soil-related environment requires careful experimental control in order to maintain bioactivities and avoid obtaining chemicals that would not be released naturally. Extraction procedures for both straw and soil samples were carried out under very mild conditions. Extracts were initially made by shaking the mixtures of sample materials and distilled water in a cold room (about -6°C) for predetermined period of time (see procedures on p.24 and p.27). The crude extracts were filtered and centrifuged to obtain clear aqueous solutions before lyophilization. The lyophilized dry extracts were partitioned by extracting sequentially with methanol, methylene chloride, chloroform, and finally distilled water. All extracts, including crude aqueous extracts, were then bioassayed by testing their effects on germination and early seedling growth of wheat seeds (see procedure on p.29). The final separation and identification of active biochemicals was performed using the CGC/MS/DA system. Both quantitative and qualitative measurements were undertaken throughout this study.

2. Apparatus

a) Shaker: G24 Environmental Incubator Shaker, New Brunswick Scientific Co., Inc., Edison, NJ, U.S.A.

b) Centrifuge: Sorvall Superspeed RC2-B, automatic

refrigerated centrifuge, Irving, TX, U.S.A.

c) Lyophilizer: Virtis Equipment Model 10-MR-ST, The Virtis Company, Gardiner, NY. Hand-built and assembled lyophilizer, utilizing Pyrex 4-port suction device with built-in cold finger; cooled with a Cryocool CC-60 from Neslab Instruments, and utilizing a low vacuum pump.

d) Balance: Semimicro analytical balance, Ainsworth Type 24N, WM. Ainsworth and Sons. Inc., Denver, CO, U.S.A.

e) Incubator: Precision Model 805, Range 5 °C to 50 °C, Precision Scientific Corp., Chicago, IL, U.S.A.

f) CGC/MS/DA System: CGC - United Technologies Packard Model 438A gas chromatography, Model 642 Recorder, Downers Grove, Illinois; MS - LKB 2091 Capillary gas chromatography/ Mass spectrometer, LKB Producter AB, Stockholm, Sweden; DA - IBM Personal Computer AT, Teknivent Model 1050 mass spectrometer data system, Teknivent Corp., St. Louis, MO. U.S.A.

g) Confirmatory Mass Spectrometry:

70/70 Mass Spectrometer, VG Analytical Limited, Wythenshawe, Manchester, England.

3. Chemical Reagent

All the organic solvents used were of Baker-Resi-Analyzed reagent grade purchased from J.T.Baker Chemical Co., Phillipsburg, NJ, U.S.A.

Diazomethane, used as a methylation reagent, was synthesized as described by Ruehle *et al.*(45), and kept at -18 °c in ethyl ether in a dark container until use.

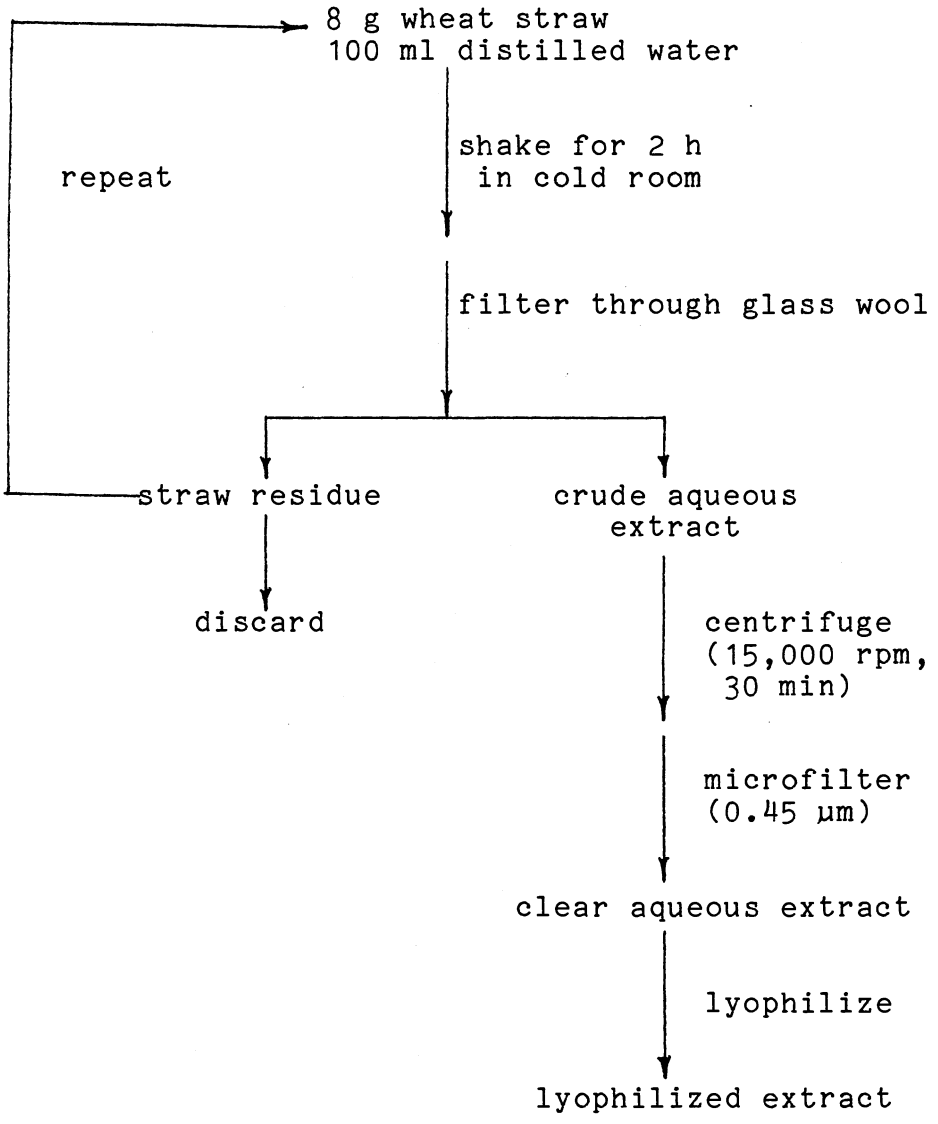
4. Straw Extraction Procedure

A mixture of 8 g straw material and 100 ml distilled water was shaken in a cold room at temperature of -6°C for 2 hours. After being filtered through glass wool to remove straw residues, the extract was centrifuged in 50-ml Teflon tubes at 15,000 rpm for 30 min and filtered again through a Millipore filter (type HA, 0.45 µm/porosity, Millipore Corporation, Bedford, Massachusetts, U.S.A.). Each straw sample was extracted twice and the two crude aqueous extracts were combined after the micro-filtration. The clear crude extract was then lyophilized to dryness and the residue weighed and stored *in vacuo*. The procedure is illustrated in Figure 1.

5. Soil Extraction Procedure

200 g of soil and 400 ml of distilled water were mixed by stirring. The pH of the mixture (pH 4.5 ± 0.2 for NT soil and pH 5.0 ± 0.2 for CT as normal pH values, respectively) was slowly adjusted to the predetermined pH by dripping in 1N NaOH (0.1N NaOH for final adjustment). The dispersed slurry was shaken gently for 48 hours in a cold room (ca -6 °C). The muddy extract was refrigerated (ca 5 °C) while the heavy soil particles settled, and afterward centrifuged and filtered through a Millipore filter. After Millipore filtration, conventional-tillage soil extracts had a pH of 7.4 ± 0.2 and no-tillage of 7.1 ± o.2 measured as final pH values in the case of basic extraction.

Figure 1. Straw Extraction Procedure.


8 g wheat straw
100 ml distilled water
shake for 2 h
in cold room
repeat
filter through glass wool
straw residue
crude aqueous
extract
discard
centrifuge
(15,000 rpm,
30 min)
microfilter
(0.45 µm)
clear aqueous extract
lyophilize
lyophilized extract

The total volume of each crude extract was divided into 3 portions: 8.0 ml for direct bioassay, 200 ml for lyophilization, and the rest about 150 ml for storage in the refrigerator. The lyophilized dry extract was weighed and stored *in vacuo*. The soil extraction procedure is illustrated in Figure 2.

6. Fractionation Procedure

The lyophilized extracts of both straw and soil were extracted sequentially with methanol, methylene chloride and chloroform. Extract residues were air dried after each extraction. Soil samples were extracted with methanol only. In each extraction, 15 ml solvent was applied and the slurry was gently heated on a hot plate, in a fume hood, with good stirring. The mixture was then filtered through a Millipore filter (type FH, 0.45 μm/porosity). A total volume of 45 ml solvent was used (3 repeats, 15 ml per each extraction), and 3 separated sub-extracts were combined. Each fraction was reduced to dryness under N_2, and prepared as equivalent grams of soil or straw per milliliter of solution for bioassay (see p. 37 and p. 38 for explanation).

After organic-solvent extraction, the residues were air dried and extracted finally with distilled water to test the completeness of isolation of bioactivities by the various organic solvent extractions. The fractionation procedure is illustrated in Figure 3.

Figure 2. Soil Extraction Procedure.

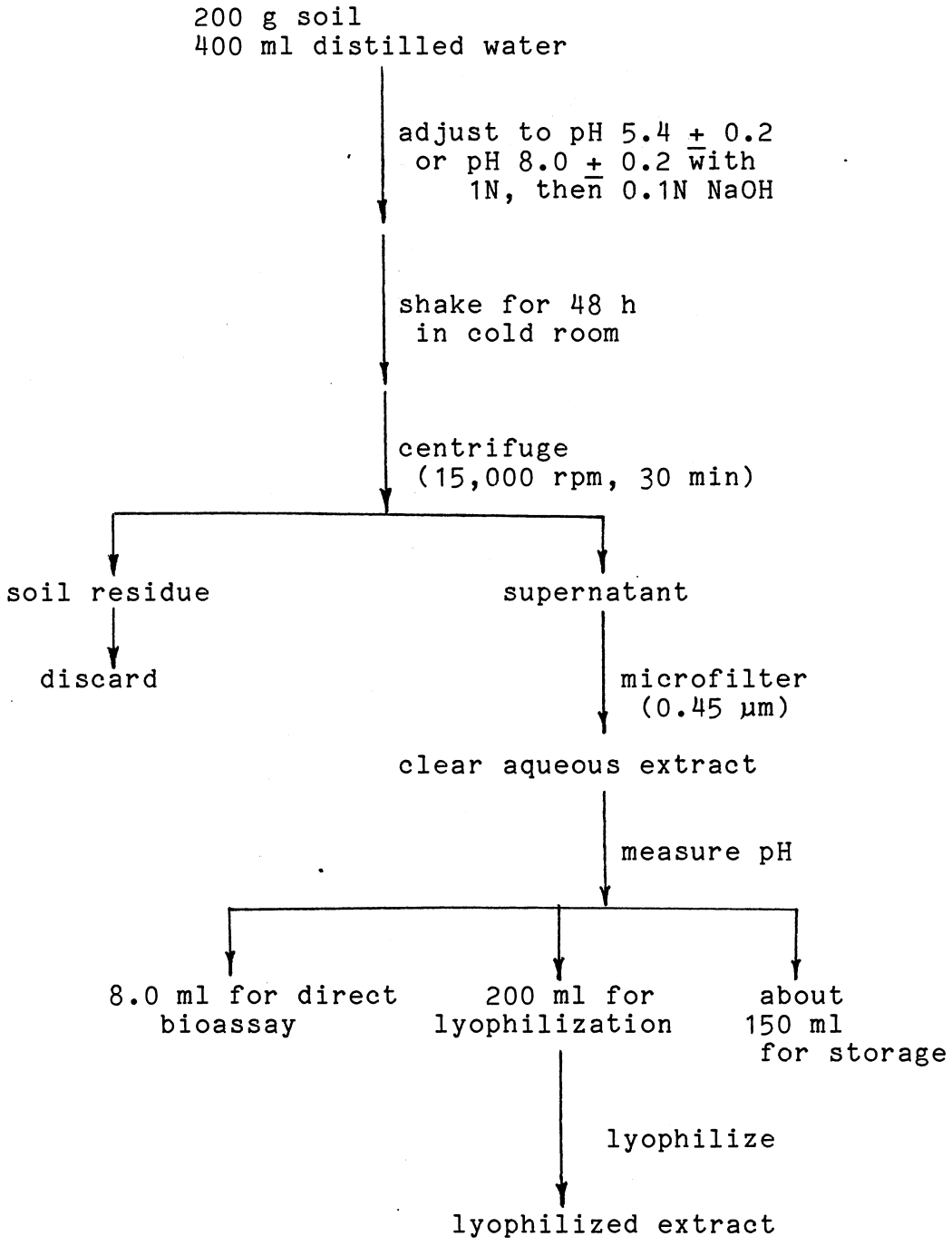
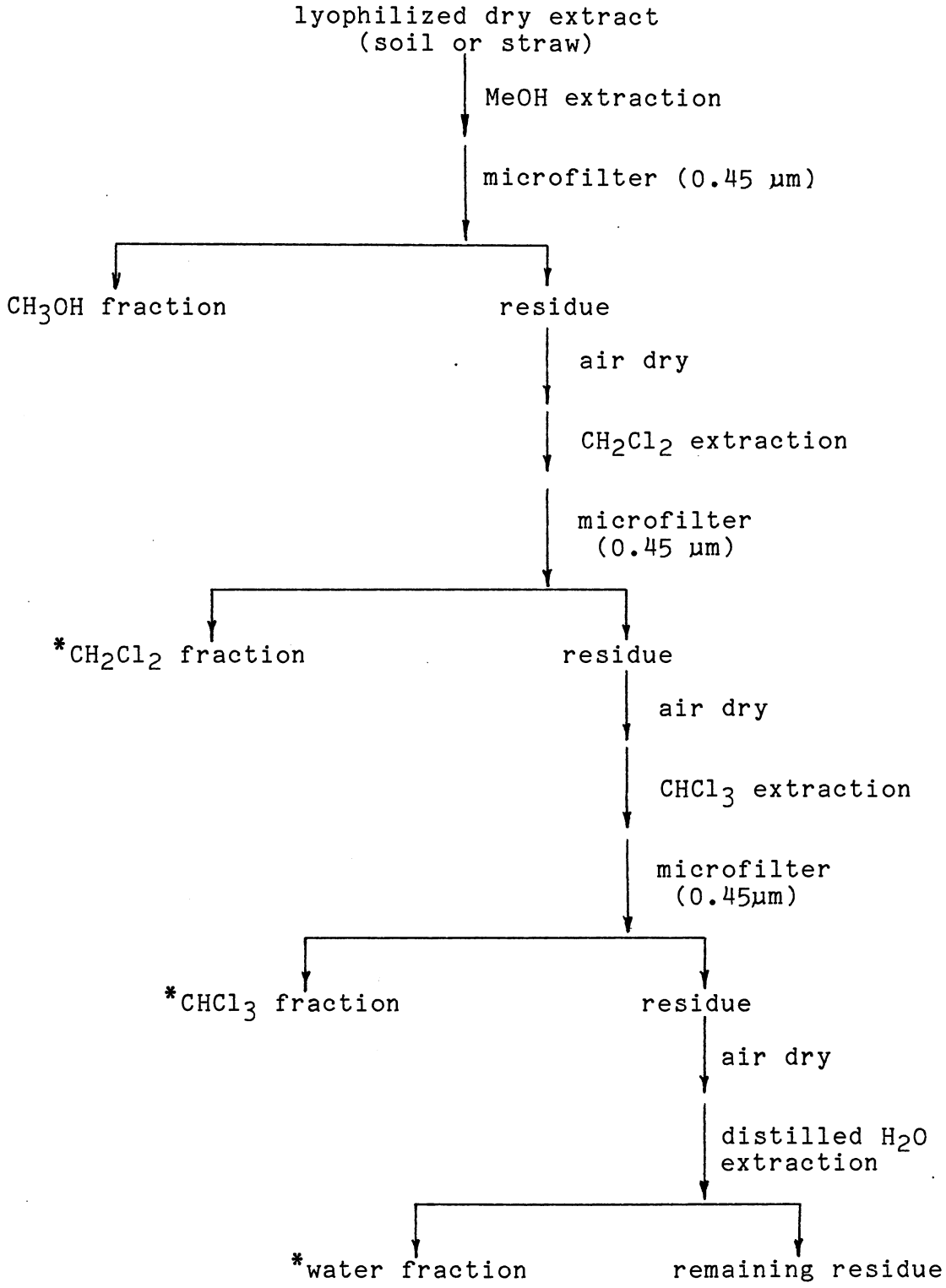

200 g soil
400 ml distilled water
adjust to pH 5.4 ± 0.2
or pH 8.0 ± 0.2 with
1N, then 0.1N NaOH
shake for 48 h
in cold room
centrifuge
(15,000 rpm, 30 min)
soil residue
supernatant
discard
microfilter
(0.45 µm)
clear aqueous extract
measure pH
8.0 ml for direct
bioassay
200 ml for
lyophilization
about
150 ml
for storage
lyophilize
lyophilized extract

Figure 3. Scheme of Fractionation Procedure.

* Soil samples were extracted with MeOH only.


lyophilized dry extract
(soil or straw)
MeOH extraction
microfilter (0.45 µm)
CH_3OH fraction
residue
air dry
CH_2Cl_2 extraction
microfilter
(0.45 µm)
*CH_2Cl_2 fraction
residue
air dry
$CHCl_3$ extraction
microfilter
(0.45µm)
*$CHCl_3$ fraction
residue
air dry
distilled H_2O
extraction
*water fraction
remaining residue

7. Bioassay Procedure

The characterization and identification of allelochemicals require sensitive bioassay methods that are relevant to the critical period of wheat growth. The method used in this study is similar to that described by McPherson and Muller (46).

a) Materials. Glass Petri dishes, 100 x 15 mm, were used as containers with two filter papers (Whatman No.1, 75 mm) forming the absorptive medium. Ten seeds of Pioneer 2157 wheat were placed between the two sheets of filter paper in a radial pattern with the micropyle end toward the center. Seeds were hand-selected for normal size and absence of damage. Pioneer 2157 is the cultivar of wheat used in the on-going field research on conservation-tillage practices. Each Petri dish, after setting the test seeds in the medium, was covered tightly with a square of kitchen-type plastic wrap before pressing the top dish cover over to reduce moisture evaporation.

b) Exposure to test samples against controls. Two sheets of filter paper can absorb 2.0 ml of water or aqueous solution for thorough saturation. In the case of aqueous solutions, 2.0 ml solution was applied directly to the filter papers with seeds between them. Distilled water was used for the controls. With organic-solvent extracts, 2 ml solution was applied to the filter paper and allowed to evaporate completely before seeds were arranged between them. Distilled water (2 ml) was then applied to permit seed germination and seedling growth. Corresponding pure

solvents were applied as controls following the same steps as those of the tests. The concentrations of the test samples were expressed as equivalent grams of wheat straw or soil per seed (p. 37 and p. 38). When potential allelochemicals were isolated from straw or soil and further identified in CGC/MS/DA analysis, standard chemicals were bioassayed by the same method as a crosscheck on the bioactivity.

c) Incubation conditions. Incubation was at 20 °C for 72 h in darkness. Preliminary trials indicated that these conditions were optimum for adequate wheat growth and retardation of mold growth.

d) Replication. Four Petri dishes, each containing ten seeds, were used for each test, both sample and control. Controls were run with the samples in all treatments.

e) Results, parameters and measurements. The lengths of the central root and shoot of each seedling were measured. Means of each set of measurements, including controls, were calculated. The difference between sample and corresponding control was indicated by percent inhibition or stimulation compared to the control as well as standard statistical analysis (*t*-test) at 95% and 99% significance levels (47).

8. Purification and Determination of Allelochemical Structures

A CGC/MS/DA system was used for further separation and structure determination of the compounds that were tested in

the bioassays.

a) Preparation of methylated derivatives. Methylated derivatives of some extracts or compounds were prepared for better mass spectrometry performance by adding several drops of diazomethane solution to samples and shaking intermittently until the characteristic yellow color in the reaction solution had disappeared. Any remaining diazomethane was expelled by evaporating over night or under N_2 in the fume hood.

b) CGC/MS/DA analysis. The CGC/MS/DA system described earlier served for final separation and structural analysis in this study. Detailed information of this type of instrument was given by Waller and McGown (48,49).

i. Parameters and conditions of GC operation

Column: J&W DB-5 capillary column, 0.25 mm x 30 m, 0.1 μm film with 1:4 split.

Carrier gas: Helium at 30 ml/min

Detector temperature: 280 °C

Injector temperature: 280 °C

Aux. temperature: 280 °C

Stability: 1

The GC separation was carried out under the following operational parameters: initial column temperature 60°C, adjusted ± 5°C according to various solvents, kept at initial temperature for 4 min., followed by temperature programming from 60°C to 300°C at 10°C/min, column was then held at final temperature of 300°C for 20-30 min.

ii. Operation conditions of mass spectrometer

Pressure: 1×10^{-6} to 1×10^{-7} torr

Electron ionizing voltage: 20/70 ev

(for operation of the recorder it was necessary to set the electron impact voltage at 20 ev to reduce the amount of $He^{+\cdot}$, each time there was electronic switching to make sure that the spectra taken was at 70 ev.)

Trap current: 60-95 μA

Accelerating voltage: 3.5 KV

Box ampere: 30-50 μA

Filament ampere: 4.2 A

Separator Temperature: 270 °C

Multiplier setting: 500-600

Source Temperature: 260 °C

All the organic fractions of aqueous extracts of wheat straw and wheat soil that were characterized primarily on the CGC/MS/DA system. The system was calibrated with the standard mass spectrum of tris-(heptafluropropyl)-s-triazine (PCR, Research Chemical Inc.) and the Capillary Column Test Mix, DB-1 and DB-5 (J&W Scientific Inc.) before the samples were analyzed every time. A small amount of pure caffeine was also used as an internal standard.

iii. Mass Spectral Data Processing

Identification of components separated by GC was based on the comparison of unknown MS spectra with known standard MS spectra (51, 52) and interpretation of the normal fragmentation patterns (53, 54). The data analysis program

utilizing probability based searching techniques provided further information on the identification of the compounds.

CHAPTER IV

RESULTS AND DISCUSSION

A. Physical Appearances of Water Extractable Substances

1. Comparison of New Wheat Straw and Old Wheat Straw

The amount of water-extractable substance in new wheat straw was about 3 times as much as that in old wheat straw under the same extraction conditions. Data of seven separate extractions are presented in Table III.

The new-wheat-straw extract was dark and sticky compared with that of old wheat straw, which was lighter and more powdery.

These differences suggested that new wheat straw contained more compounds, some of which were released and leached into the soil during decomposition process. At least some of those compounds missing from old straw were expected to be recovered from the soil. Some of these compounds, or their breakdown products, may be phytotoxic to subsequent wheat planting. Some sugars and their derivatives were suspected to exist in fresh wheat straw. They cause the sticky consistency.

TABLE III

AMOUNT OF WATER-EXTRACTABLE SUBSTANCES
FROM 8 GRAMS RAW WHEAT STRAW

Extraction No.	Weight of Lyophilized Extracts (g)	
	Old Wheat Straw	New Wheat Straw
A	0.127	0.356
B	0.102	0.360
C	0.105	0.336
D	0.125	0.366
E	0.187	0.377
F	0.092	0.306
G	0.078	0.211
Average	0.116	0.330

Note: Letters A, B, C, etc. represent separate extractions.

2. Comparison of Acidities of Conventional-tillage Soil and No-tillage Soil

The initial pH's (pH values before shaking), final pH's (pH values after shaking) of both conventional-tillage soil and no-tillage soil extracts, and the weights of the lyophilized extracts are listed in Table IV.

TABLE IV

MEASUREMENTS OF ACIDITY, BASICITY AND QUANTITIES OF SOIL EXTRACTS (200 G SOIL EXTRACTED)

Sample	Initial pH of Slurry	Final pH of Extracts	Lyophilized Extracts (g)
Acidic [a]CT extract	5.43	5.60	0.796
Acidic [b]NT extract	5.18	5.08	*0.541
Basic [a]CT extract	8.04	7.50	0.507
Basic [b]NT extract	8.04	7.17	**0.672

[a] CT - Conventional-tillage soil.

[b] NT - No-tillage soil.

* Lyophilized sample was very sticky. Probably half of the total extract was lost because of being stuck on the flask.

** Lyophilized sample was sticky. Some of the extract was stuck on the flask.

The natural soil is an acidic buffer system with pH 4.2-5.0. The soil buffer system was so strong that about 2.3 ml of 1N NaOH had to be added into CT soil slurry (soil:water = 200 g:400 ml) to adjust the pH to 8.0 $\pm$ 0.2; correspondingly, and about 2.8 ml of 1N NaOH had to be added for NT soil. Higher acidity of NT soil extracts (Table IV) compared to that of CT soil extracts under the same extraction conditions indicated that more acidic compounds were present in no-tillage soil. The odor and sticky physical appearance of NT soil extract were similar to that of new wheat straw. This similarity was consistent with the conjecture that some chemicals escape from straw into the soil. These compounds may act as allelopathic agents.

B. Bioassay of Old and New Wheat Straw Extracts

As described earlier (p. 25), the bioassay technique was designed to test the effects of various wheat straw and soil extracts on the early growth and development of wheat seedlings. In order to assay the biological activity with respect to the amount of sample material, a series of concentrations of straw and soil extracts were tested. The concentrations were expressed as equivalent grams of raw sample material (straw or soil) per milliliter of bioassay solution (g/ml). This expression can be explained as follows. If the total amount of straw extracted was 8 grams and a total volume of 190 ml crude aqueous extract was obtained, the concentration of this crude extract measured

as equivalent amount of straw per milliliter would be 8 [g straw]/190 [ml] = 0.04 [g/ml]. Thus the concentration of the direct bioassay, in which 8.0 ml of the crude aqueous extract was tested, was also 0.04 [g/ml]. The remaining 182 ml crude aqueous extract was lyophilized and 0.300 grams of dry extract were obtained after the lyophilization. If 0.100 grams of lyophilized extract is weighed out and dissolved in 8.0 ml distilled water for bioassay, the concentration of this solution is 0.100 [g lyoph.ext.] / 8.0 [ml] = 0.0125 [g lyoph.ext./ml]. Thus, the concentration expressed as equivalent amount of straw per milliliter would be

$$0.0125\ [\text{g lyoph.ext./ml}] / 0.300\ [\text{g lyoph. ext.}] \times (182/190)\ [\text{ml/ml}] \times 8\ [\text{g straw}] = 0.32\ [\text{g straw/ml}]$$

The concentrations of organic solvent fractions were calculated in the same manner on the basis of the amount of lyophilized extract that corresponded to grams of straw that was extracted.

The bioassay was conducted on 40 seeds for each extract. Bioassay tests of wheat straw extracts and fractions on growth of wheat seedlings are summarized in Table V and Table VI.

As the data showed in Table V and Table VI, the inhibitory biological activity of old wheat straw was present only in the methanol fraction, whereas that of new wheat straw was in the methanol, methylene chloride, chloroform, final water fractions, and in the remaining residue. In the bioassays of old wheat straw, no

TABLE V

BIOASSAY RESULTS OF OLD WHEAT STRAW, COLLECTED IN JUNE, 1985

Years 1982, 1983, 1984 residues of straw were observed when collected as a mixed straw sample.

Testing Sample	Conc. of Straw Ext. Solution (g/ml)	Root Length (mm)	Shoot Length (mm)	Inhib. (-%) Stimu. (+%) Root	Shoot
Crude aq. ext.	0.05	33.8 ± 2.5[a] *(25.5 ± 2.3)	10.3 ± 0.6 *(9.1 ± 0.7)	+33	+12
Lyoph. ext.	0.80	11.4 ± 0.7[b] *(33.4 ± 1.7)	9.0 ± 0.4[b] *(12.8 ± 0.5)	-66	-30
CH_3OH fract.	0.80	7.8 ± 0.7[b] *(22.8 ± 2.3)	4.2 ± 0.4[b] *(7.2 ± 0.5)	-66	-42
CH_2Cl_2 fract.	1.60	28.6 ± 1.9 *(29.5 ± 1.7)	11.0 ± 0.6 *(10.0 ± 0.5)	- 3	+10
$CHCl_3$ fract.	1.60	27.8 ± 2.7 *(27.4 ± 2.4)	10.3 ± 0.6 *(10.1 ± 0.8)	+ 1	+ 2
Final H_2O fract.	0.80	29.0 ± 1.6 *(24.7 ± 2.0)	10.5 ± 0.2 *(9.0 ± 0.5)	+17	+17

* Controls (see p.30 for further explanation).

[a] Significantly different from control at 95% level of confidence or better (t-test).

[b] Significantly different from control at 99% level of confidence or better (t-test).

TABLE VI

BIOASSAY RESULTS OF NEW WHEAT STRAW, COLLECTED IN JUNE, 1985

Testing Sample	Conc. of Straw Ext. Solution (g/ml)	Root Length (mm)	Shoot Length (mm)	Inhib. (-%) Stimu. (+%) Root	Shoot
Crude aq. ext.	0.05	31.0 ± 1.5 *(25.5 ± 2.3)	9.0 ± 0.5 *(9.1 ± 0.7)	+22	- 2
Lyoph. ext.	0.80	7.0 ± 0.6[b] *(33.4 ± 1.7)	5.2 ± 0.3[b] *(12.8 ± 0.5)	-79	-59
CH_3OH fract.	0.80	6.9 ± 0.6[b] *(28.7 ± 1.6)	5.1 ± 0.3[b] *(10.1 ± 0.5)	-76	-50
CH_2Cl_2 fract.	1.60	18.2 ± 1.8[b] *(26.7 ± 1.7)	7.5 ± 0.6 *(8.4 ± 0.4)	-32	-11
$CHCl_3$ fract.	1.60	17.8 ± 1.5 *(21.0 ± 2.8)	7.5 ± 0.5 *(7.8 ± 0.7)	-15	- 4
Final H_2O fract.	0.80	13.0 ± 0.8[b] *(23.7 ± 2.3)	4.4 ± 0.3[b] *(7.7 ± 0.5)	-46	-43
**Remaining residue	--	17.5 ± 2.1 *(23.7 ± 2.3)	5.7 ± 0.5[a] *(7.7 ± 0.5)	-26	-26

* Controls (see p.30 for further explanation).

** Remaining residue consisted of all that remained after fractionation.

[a] Significantly different from control at 95% level of confidence or better (t-test).

[b] Significantly different from control at 99% level of confidence or better (t-test).

significant amount of toxicity was found in methylene chloride, chloroform and final water fractions, and there was too little residue remained for bioassay tests. The results indicated that some toxic compounds of new straw that are soluble in methylene chloride and/or chloroform but insoluble or less soluble in methanol are missing in old wheat straw. As mentioned earlier, the old wheat straw was a mixture of three previous years' that were accumulated on the surface of the field, so many compounds must have been released into the soil. The toxicity of the lyophilized aqueous extract was not equal to the sum of the toxicities of the fractions since they exhibit a synergistic effect instead.

Bioassays of crude aqueous extracts of both old and new wheat straw stimulated wheat seedling growth and development. This material was in concentrations about one order of magnitude lower than the organic solvent-dissolved substances, and probably reflected one of the most important properties of allelochemicals, i.e., allelochemicals may act as growth stimulants at very low concentrations, but become inhibitors when they are present at higher concentrations.

C. Bioassay of Conventional-Tillage and No-Tillage Wheat Soil Extracts

The normal pH was about 5.0 for CT soil and 4.5 for NT soil. The extraction of soil was carried out under acidic (pH 5.4 $\pm$ 0.2) and basic (pH 8.0 $\pm$ 0.2) conditions in order to compare the possible differences in biological activities

and allelochemical composition of the extracts (all pH values were recorded 2 min after the readings were completely stable because the soil solutions showed fairly strong buffer activity). The reason for extracting the soil under these conditions rather than neutral conditions, as used in straw extractions, is due to the relatively lower concentrations of water-extractable chemicals in soil than in straw. These conditions, being relatively mild, were not considered to be harsh treatments. In fact they were considered to most closely approximate the natural environment.

Solutions of aqueous soil extracts were adjusted to pH 7.0 $\pm$ 0.2 for bioassays. The bioassay results of soil extracts are shown in Table VII and Table VIII. Crude aqueous extracts were all stimulatory as were those of wheat straw. The only exception was the acidic extract of CT June soil, which showed no activity, neither inhibitory nor stimulatory.

These results showed again that allelochemicals may exhibit stimulatory effects on plant growth rather than inhibition at low concentrations, and the stimulation may also increase with increasing concentrations within a low concentration range until a certain point of concentration was reached (see Fig. 4). Figure 4 is a graph of root growth versus concentration of the soil extracts. Shoot growth data is not presented in the graph because of the less sensitive response of shoot growth against toxins. This phenomenon was also observed by Kimber (27, 28).

TABLE VII

BIOASSAY RESULTS OF CONVENTIONAL-TILLAGE WHEAT SOIL, COLLECTED IN JUNE, 1985

Testing Sample	Conc. of Soil Ext. Solution (g/ml)	Root Length (mm)	Shoot Length (mm)	Inhib. (-%) Stimu. (+%) Root	Shoot
A. Acidic Extraction at pH 5.4 ± 0.2					
Crude aq. ext.	0.55	25.4 ± 1.4 *(25.6 ± 1.7)	7.8 ± 0.4 *(8.1 ± 0.4)	- 1	- 4
Lyoph. ext.	0.55	37.0 ± 1.4 *(36.1 ± 0.3)	15.2 ± 0.5[b] *(12.9 ± 0.5)	+ 2	+18
Lyoph. ext.	1.70	39.6 ± 1.6 *(36.1 ± 0.3)	13.8 ± 0.4 *(12.9 ± 0.5)	+10	+ 7
Lyoph. ext.	2.85	45.5 ± 2.1[b] *(36.1 ± 1.3)	13.8 ± 0.6 *(12.9 ± 0.5)	+26	+ 7
Lyoph. ext.	3.60	25.0 ± 1.2[a] *(20.6 ± 1.1)	7.9 ± 0.3 *(7.9 ± 0.3)	+21	0
Lyoph. ext.	4.50	24.3 ± 1.2[a] *(20.6 ± 1.1)	7.6 ± 0.4 *(7.9 ± 0.3)	+18	- 4
Lyoph. ext.	7.80	24.1 ± 1.3 *(23.9 ± 1.3)	9.0 ± 0.4 *(8.7 ± 0.4)	+ 1	+ 3
CH_3OH fract.	7.80	28.4 ± 1.4 *(28.0 ± 1.1)	9.5 ± 0.5 *(8.7 ± 0.4)	+ 1	+ 9

TABLE VII (Continued)

Testing Sample	Conc. of Soil Ext. Solution (g/ml)	Root Length (mm)	Shoot Length (mm)	Inhib. (-%) Stimu. (+%) Root	Shoot
B. Basic Extraction at pH 8.0 ± 0.2					
Crude aq. ext.	0.55	35.6 ± 1.7[b] *(23.9 ± 1.3)	10.2 ± 0.5[a] *(8.7 ± 0.4)	+49	+17
Lyoph. ext.	0.55	38.6 ± 1.1 *(36.1 ± 1.3)	15.0 ± 0.4[a] *(12.9 ± 0.5)	+ 7	+16
Lyoph. ext.	1.60	36.0 ± 1.0 *(36.1 ± 1.3)	14.2 ± 0.5 *(12.9 ± 0.5)	0	+10
Lyoph. ext.	2.70	31.3 ± 1.7[a] *(36.1 ± 1.3)	13.1 ± 0.6 *(12.9 ± 0.5)	-13	+ 2
Lyoph. ext.	4.00	18.2 ± 1.2[a] *(20.6 ± 1.1)	6.7 ± 0.4[a] *(7.9 ± 0.3)	-16	-15
Lyoph. ext.	7.80	18.3 ± 0.8[b] *(23.9 ± 1.3)	8.5 ± 0.3 *(8.7 ± 0.4)	-23	- 2
CH_3OH fract.	7.80	15.7 ± 1.4[b] *(23.7 ± 2.5)	8.3 ± 0.7 *(9.3 ± 0.8)	-34	-10

* Controls (see p.41 for further explanation).

[a] Significantly different from control at 95% level of confidence or better (t-test).

[b] Significantly different from controls at 99% level of confidence or better (t-test).

TABLE VIII

BIOASSAY RESULTS OF NO-TILLAGE WHEAT SOIL, COLLECTED IN JUNE, 1985

Testing Sample	Conc. of Soil Ext. Solution (g/ml)	Root Length (mm)	Shoot Length (mm)	Inhib. (-%) Stimu. (+%) Root	Inhib. (-%) Stimu. (+%) Shoot
A. Acidic Extraction at pH 5.4 ± 0.2					
Crude aq. ext.	0.55	28.4 ± 1.7 *(26.3 ± 1.3)	7.9 ± 0.2 *(8.3 ± 0.2)	+ 8	- 5
Lyoph. ext.	0.55	35.5 ± 2.0 *(33.5 ± 1.4)	11.5 ± 0.6 *(10.9 ± 0.4)	+ 6	+ 5
Lyoph. ext.	1.10	24.6 ± 1.5 *(22.8 ± 1.5)	8.1 ± 0.3 *(7.4 ± 0.2)	+ 8	+ 9
Lyoph. ext.	1.70	26.9 ± 1.4[a] *(22.8 ± 1.5)	8.8 ± 0.3[b] *(7.4 ± 0.2)	+18	+19
Lyoph. ext.	4.00	23.7 ± 0.8[a] *(20.6 ± 1.1)	7.3 ± 0.3 *(7.9 ± 0.3)	+15	- 8
Lyoph. ext.	6.25	20.4 ± 1.0 *(20.6 ± 1.1)	7.7 ± 0.4 *(7.9 ± 0.3)	- 1	- 8
Lyoph. ext.	7.85	29.3 ± 1.3 *(31.0 ± 1.0)	8.7 ± 0.4[a] *(10.1 ± 0.4)	- 5	-14
CH_3OH fract.	0.55	24.8 ± 1.4 *(26.0 ± 1.2)	8.3 ± 0.4 *(8.9 ± 0.5)	- 5	- 7
CH_3OH fract.	1.10	23.3 ± 1.9 *(26.0 ± 1.2)	7.6 ± 0.5 *(8.9 ± 0.5)	-10	-15

TABLE VIII (Continued)

Testing Sample	Conc. of Soil Ext. Solution (g/ml)	Root Length (mm)	Shoot Length (mm)	Inhib. (-%) Stimu. (+%) Root	Inhib. (-%) Stimu. (+%) Shoot
B. Basic Extraction at pH 8.0 ± 0.2					
Crude aq. ext.	0.55	22.8 ± 1.1[a] *(18.5 ± 1.5)	7.9 ± 0.4[a] *(6.4 ± 0.4)	+23	+23
Lyoph. ext.	0.55	23.0 ± 1.2[a] *(18.5 ± 1.5)	8.5 ± 0.3[b] *(6.4 ± 0.4)	+24	+33
Lyoph. ext.	1.70	18.4 ± 0.8 *(18.5 ± 1.5)	7.8 ± 0.3[a] *(6.4 ± 0.4)	0	+22
Lyoph. ext.	2.80	17.6 ± 0.7 *(18.5 ± 1.5)	7.8 ± 0.2[a] *(6.4 ± 0.4)	- 5	+22
Lyoph. ext.	4.00	19.2 ± 1.1 *(20.6 ± 1.1)	6.4 ± 0.4[b] *(7.9 ± 0.3)	-12	-19
Lyoph. ext.	6.50	17.7 ± 0.8 *(20.6 ± 1.1)	6.0 ± 0.4[b] *(7.9 ± 0.3)	-14	-24
Lyoph. ext.	7.85	24.4 ± 1.3[b] *(31.0 ± 1.0)	8.6 ± 0.4[b] *(10.1 ± 0.4)	-21	-15
CH_3OH fract.	0.55	23.9 ± 0.9 *(22.9 ± 1.4)	8.6 ± 0.2[a] *(7.3 ± 0.4)	+ 4	+18
CH_3OH fract.	2.80	22.2 ± 1.2 *(22.9 ± 1.4)	7.5 ± 0.3 *(7.3 ± 0.4)	- 3	+ 2
CH_3OH fract.	7.85	17.9 ± 1.3[a] *(22.9 ± 1.4)	7.2 ± 0.4 *(7.3 ± 0.4)	-22	- 1

* Controls (see p.41 for further explanation).

[a] Significantly different from control at 95% level of confidence or better (t-test).

[b] Significantly different from control at 99% level of confidence or better (t-test).

Figure 4. Wheat Seedling Growth as a Percent of Control vs. Concentrations of the Soil Extracts, Bioassay Results of June, 1985 Conventional-tillage and No-tillage Soil Extracts.

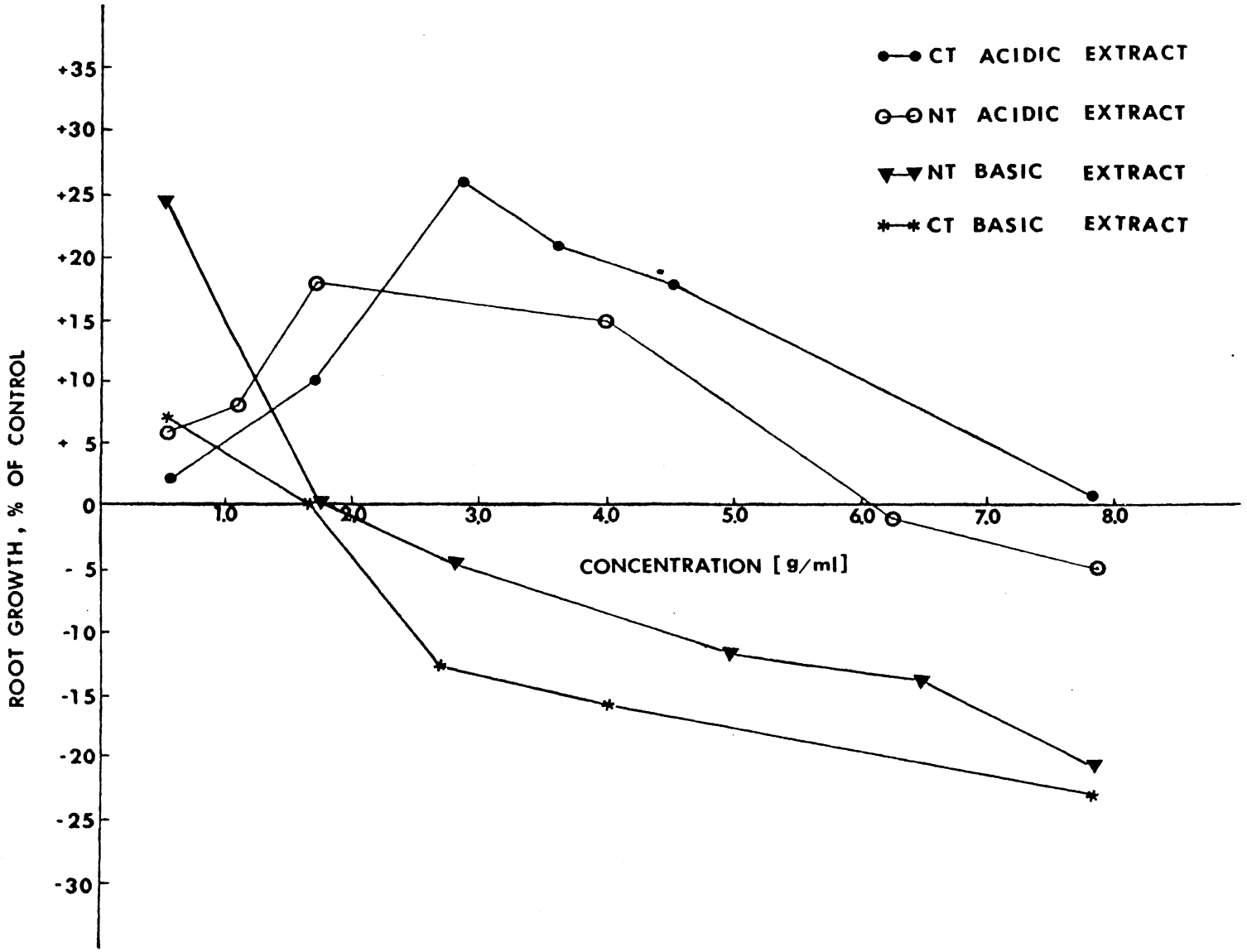

CT ACIDIC EXTRACT
NT ACIDIC EXTRACT
NT BASIC EXTRACT
CT BASIC EXTRACT
ROOT GROWTH, % OF CONTROL
+35
+30
+25
+20
+15
+10
+ 5
0
- 5
-10
-15
-20
-25
-30
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
CONCENTRATION [g/ml]

Figure 4 also indicates that after exceeding such particular concentrations the biological activities are gradually converted to less stimulatory or to inhibitory ones while the concentration increased continuously. The results may imply existence of the so-called threshold concentrations of allelochemicals that are sometimes mentioned in allelopathy studies.

It can also be seen (Table VII and Table VIII) that extracts obtained in basic extractions were more inhibitory than those at the same concentrations obtained in acidic extractions. As demonstrated in Fig. 4, for the acidic extracts (June soils of both conventional-tillage and no-tillage plots) a maximum of 17%-26% stimulatory effect was obtained at lower concentrations (about 0.5-3.0 g soil/ml) and an inhibitory action started to appear when the concentration was raised to about 6.0-8.0 g soil/ml, while for the basic extracts inhibition started at much lower concentrations (about 1.75 g soil/ml) and reached to about -22% inhibition at concentration of 8.0 g soil/ml. The results implied that more chemical compounds, at least more allelopathic chemicals, were released from the soil matrix under slightly basic conditions. Many allelochemicals may be tightly bonded to clay particles, possibly through noncovalent hydrophilic and hydrophobic bonds, and are not easily released under neutral or acidic conditions. Under slightly basic conditions, however, the hydrophilic bonds, such as hydrogen bonds, or other chemical linkages among biological chemicals and clay particles break down and the

chemicals are released.

The biological activities resulted from the combined effects of heterogeneous organic matter in the soil. The mode of the action cannot be determined because of the complexity of the soil system. We know very little about individual concentrations of phytotoxic compounds and their combinations, as well as the total soil microbial activity.

As indicated earlier, the concentrations of soil extracts were expressed as equivalent to grams of soil per milliliter of bioassay solution [g/ml]. Although the concentrations were increased from 0.55 g/ml up to about 2.43 g/ml, the equivalent amount of soil presented to each seed in the bioassays was still less than the soil mass in the natural seedling environment. Wheat seeds planted in the natural soil environment are surrounded by more soil mass and are therefore exposed to more organic matter. Since significant inhibitions were indicated in the bioassays at moderate concentrations, it can be expected that more serious allelopathic effects on wheat seedling development may occur in the normal field environment.

The comparison of allelopathic activities between June soil of the conventional-tillage plot and the no-tillage plot can be estimated from the bioassay results presented in Table VII, Table VII and Fig. 4. The acidic extracts were basically stimulatory within the concentration range tested. The basic extracts of conventional-tillage soil were more inhibitory than those of no-tillage soil at the same concentrations. It should be stressed that the actual

allelopathic effects shown in the field were not necessarily reflected by the bioassay results of the June soils. Accumulated records indicated that, as an average, growth of wheat plants in conventional-tillage plots was better than those in no-tillage plots in early spring, while no significant difference in grain yield between these two plots (Appendixes A and B). Bioassays of aqueous extracts tested the total toxicities of the water-soluble extracts. Besides the organic substances that may cause the phytotoxicities, there were other factors that need to be considered.

D. Capillary Gas Chromatography/Mass Spectrometer/Data Analysis

The organic fractions of wheat straw and soil extracts were primarily analyzed using the LKB-2091 mass spectrometer, CGC/MS/DA system previously described. The mass spectral data were obtained under the operation conditions indicated earlier. The methanol fractions were analyzed directly first, but the spectra were unsatisfactory owing to extremely poor separation. They were, therefore, methylated for better CGC/MS performance.

A problem of silicone compounds bleeding from the column was observed at higher temperatures. The background signals, which came from the column compounds referred to as column bleed, were minimized as accurately as possible by a computerized background subtraction technique in the data processing program. Any shifts in mass units caused by the

instrument's temporarily drifting during the runs were observed immediately in the spectra of the internal standard (pure caffeine), which were referred to later for necessary corrections in data interpretation. Therefore, even though the raw mass spectra of the unknowns obtained from the CGC/MS/DA system did not always exactly match their corresponding standard spectra, the deviations could be reduced or corrected to negligible differences in most cases.

Confirmatory Mass Spectrometry Analysis Identical samples were analyzed on a 70/70 Mass Spectrometer at 70 electron volts by VG Analytical Limited, Wythenshawe, Manchester, England. The spectral data obtained corresponded very well with those obtained from CGC/MS/DA system.

Mass Spectral Data As Described earlier, the identification of components analyzed by mass spectrometry were based on the comparison of the spectra of unknown with that of standard spectra, and the computer searching information. Such probability-based information, however, was taken into account only when the given confidence parameters, i.e., the fitness and the quality of the match, were at the same or higher level as those given to the internal standard added to the samples, usually above 60% for fitness and 98% for quality. Such a procedure of identification assured a high certainty of correct identification.

All spectra are presented as pairs: fractions of the aqueous new wheat extract vs. corresponding fractions of the aqueous old wheat straw extracts, and fraction of the

aqueous conventional-tillage soil extract vs. corresponding fraction of the aqueous no-tillage soil extracts. The differences in chemical composition of each pair of samples are compared and discussed in relation with their apparent allelopathic effects. The possible allelochemically related linkage between wheat straw and soil are also discussed.

The computer reconstructed total ion current chromatograms of the methylated methanol fraction of new wheat straw and the methylated methanol fraction of old straw were shown in Fig. 5 and Fig. 6, respectively. The peak-by-peak processed mass spectral data indicated that most of the components were the same in both straw extracts. The retention times of the corresponding compounds exited from the column are very close as indicated by the corresponding numbers. More chemicals were extracted from new wheat straw. Of the approximately 43 compounds of which mass spectra were taken, many were plasticizers (discussed later) and hydrocarbons. Methyl esters of four short-chain dicarboxylic acids were identified at lower retention times. They are dimethyl malonic acid (peak 5), dimethyl fumaric acid (peak 8), dimethyl succinic acid (peak 9) and dimethyl malic acid (peak 12). The obtained mass spectra of these components along with their standard spectra are shown in Figures 7, 8, 9 and 10, respectively. The discussion of possible biological activities of carboxylic acids focuses on their free acid forms as they occur naturally. They were converted into the methyl esters for easier handling in the mass spectrometry analysis.

Figure. 5 Reconstructed Total Ion Current Chromatogram of Methylated Methanol Fraction of New Wheat Straw.

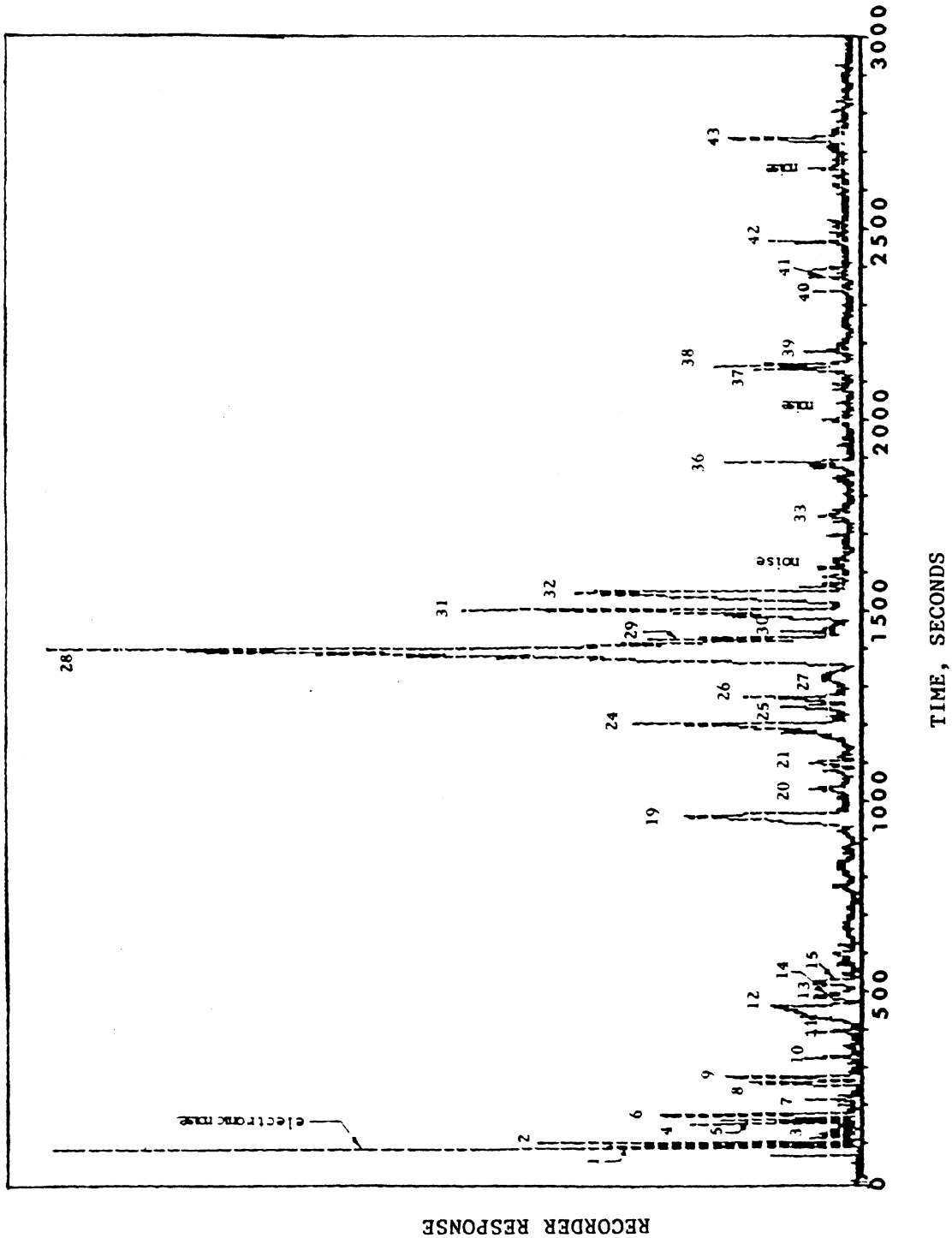

RECORDER RESPONSE
TIME, SECONDS
0
500
1000
1500
2000
2500
3000
electronic noise
noise

Figure 6. Reconstructed Total Ion Current Chromatogram of Methylated Methanol Fraction of Old Wheat Straw. (Peaks labeled as 32' represent the same compound as that labeled by peak 32 according to the mass spectral data).

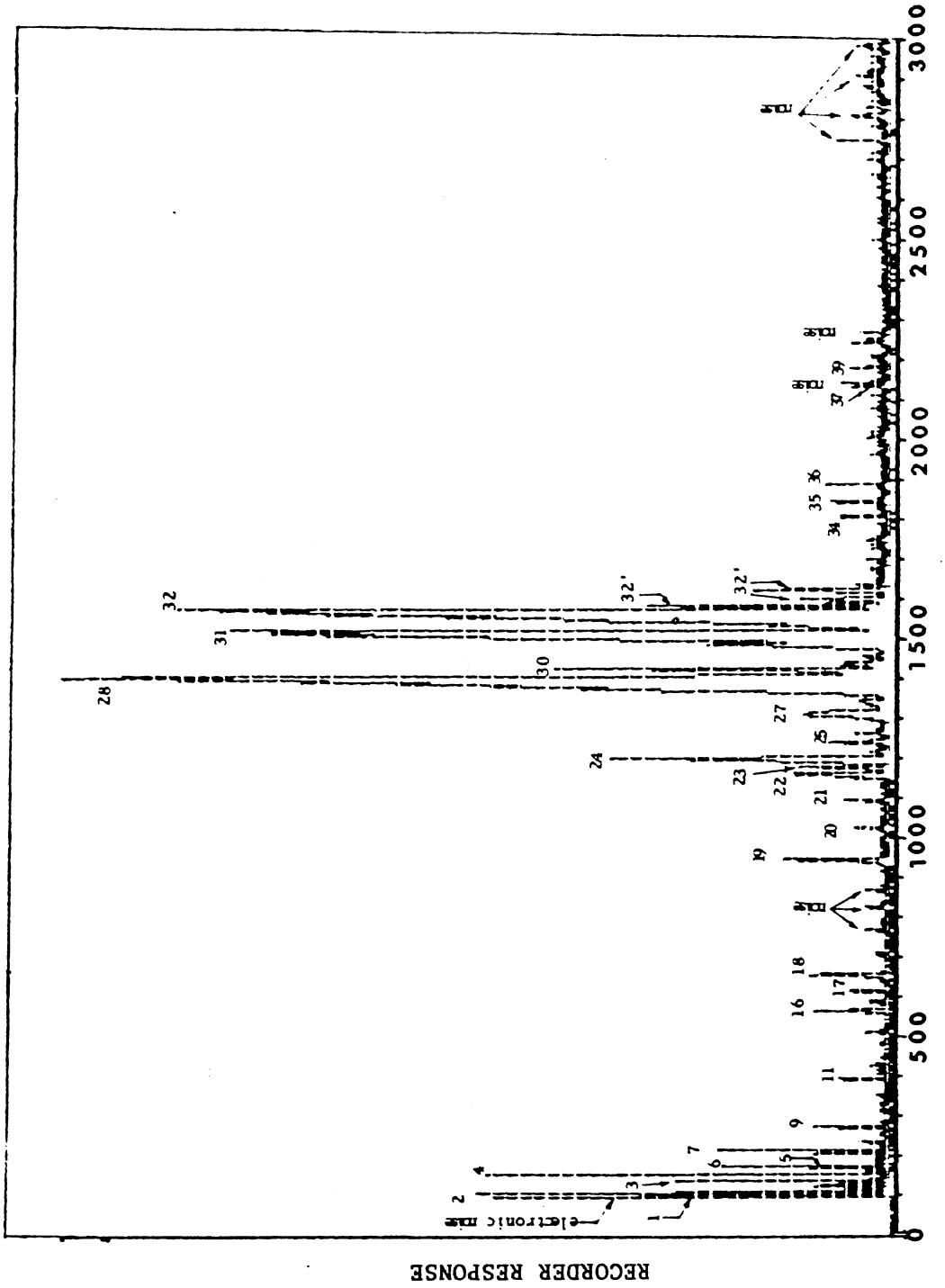
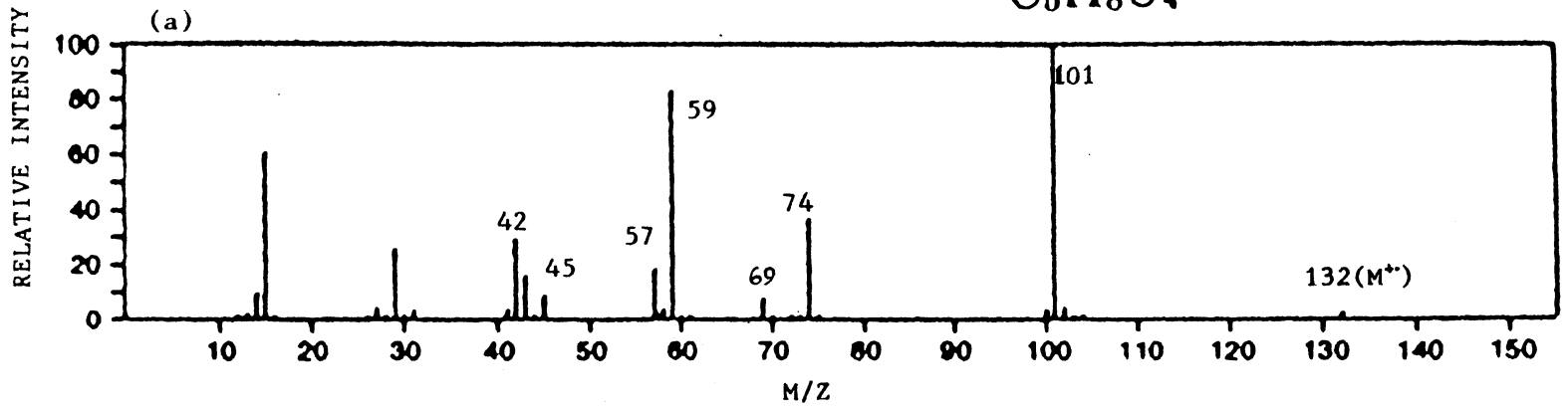

RECORDER RESPONSE
TIME, SECONDS
0
500
1000
1500
2000
2500
3000

Figure 7. (a) Standard Mass Spectrum of Malonic Acid, Dimethyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Malonic Acid, Dimethyl Ester, Corresponding to Peak 5. (Sample: methylated methanol fraction of new wheat straw.)

Malonic acid, dimethyl ester

$C_5H_8O_4$



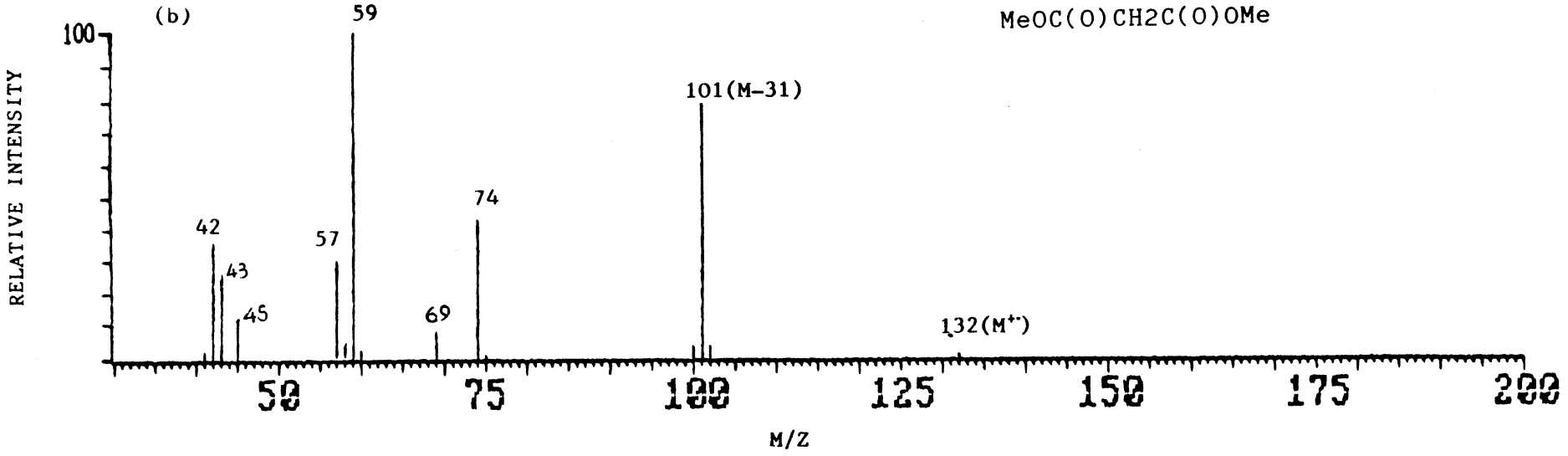
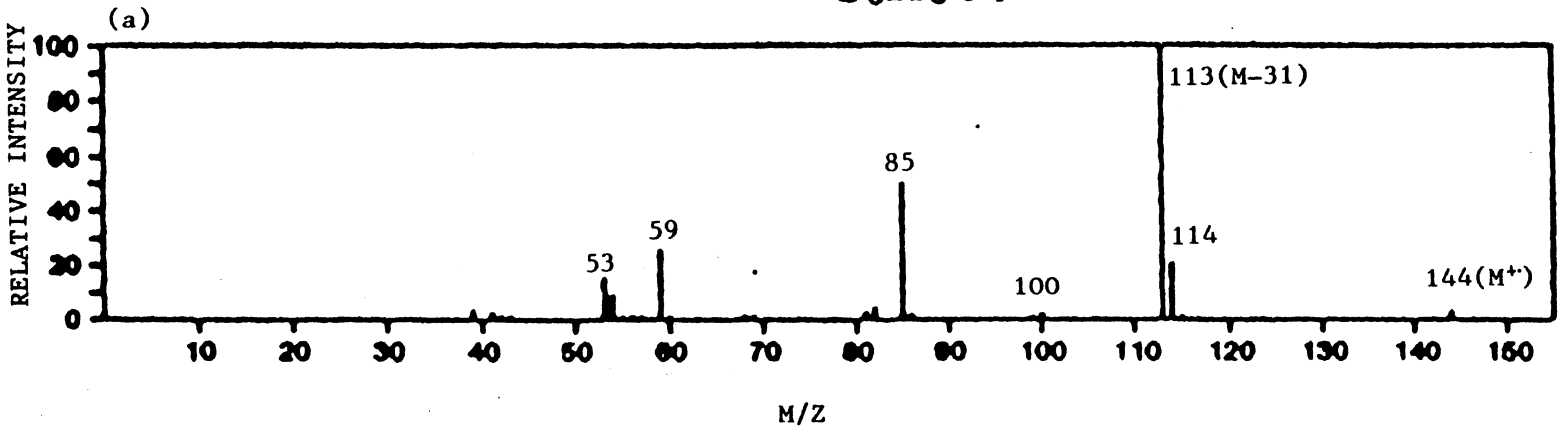


Figure 8. (a) Standard Mass Spectrum of Fumaric Acid, Dimethyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Fumaric Acid, Dimethyl Ester, Corresponding to Peak 8. (Sample: methylated methanol fraction of new wheat straw.)

Fumaric acid, dimethyl ester

$C_6H_8O_4$



MeOC(O)CH=CHC(O)OMe

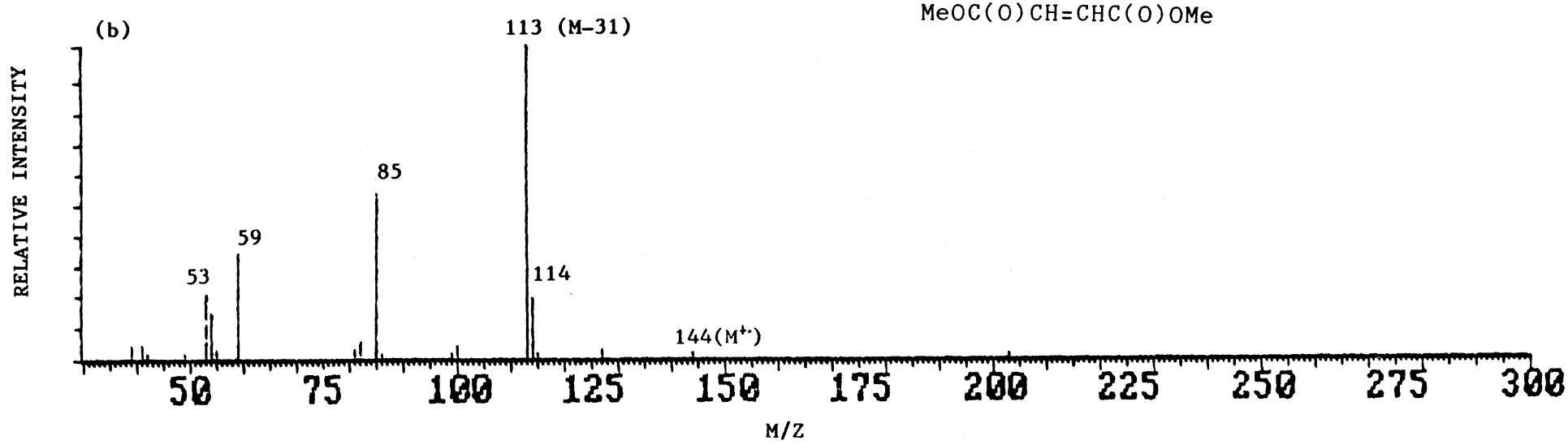
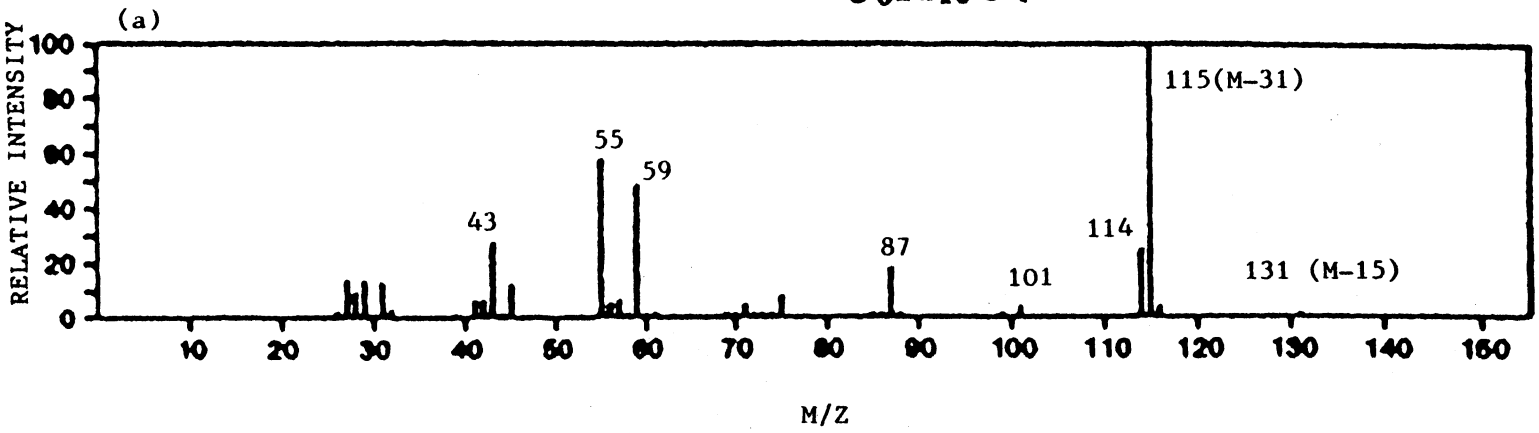


Figure 9. (a) Standard Mass Spectrum of Succinic Acid, Dimethyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Succinic Acid, Dimethyl Ester, Corresponding to Peak 9. (Sample: methylated methanol fraction of new wheat straw.)

Succinic acid, dimethyl ester $C_6H_{10}O_4$



MeC(O)CH2CH2C(O)OMe

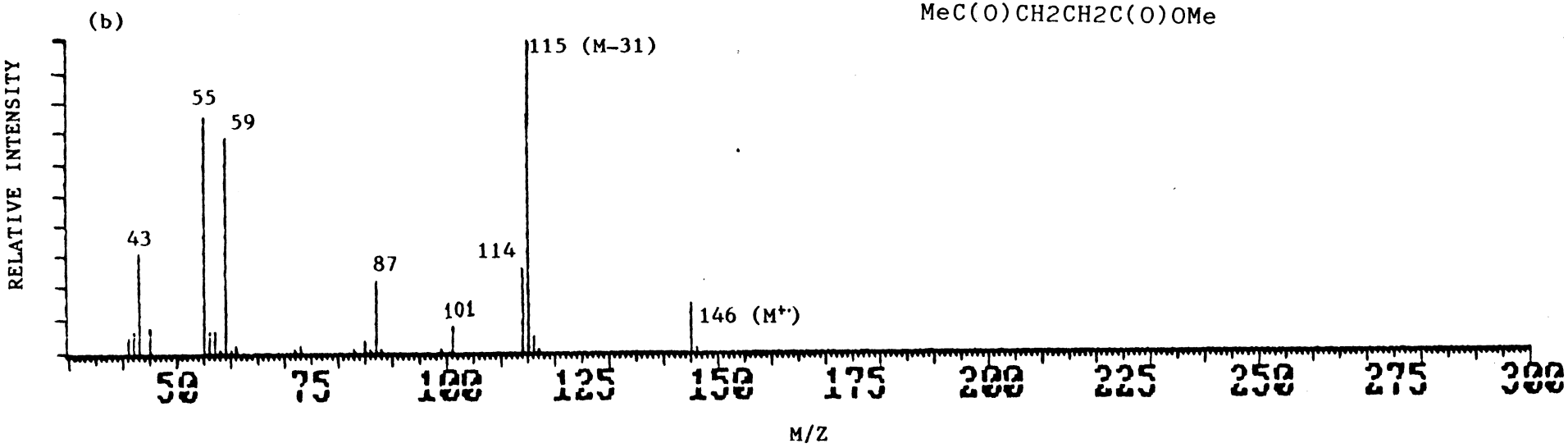
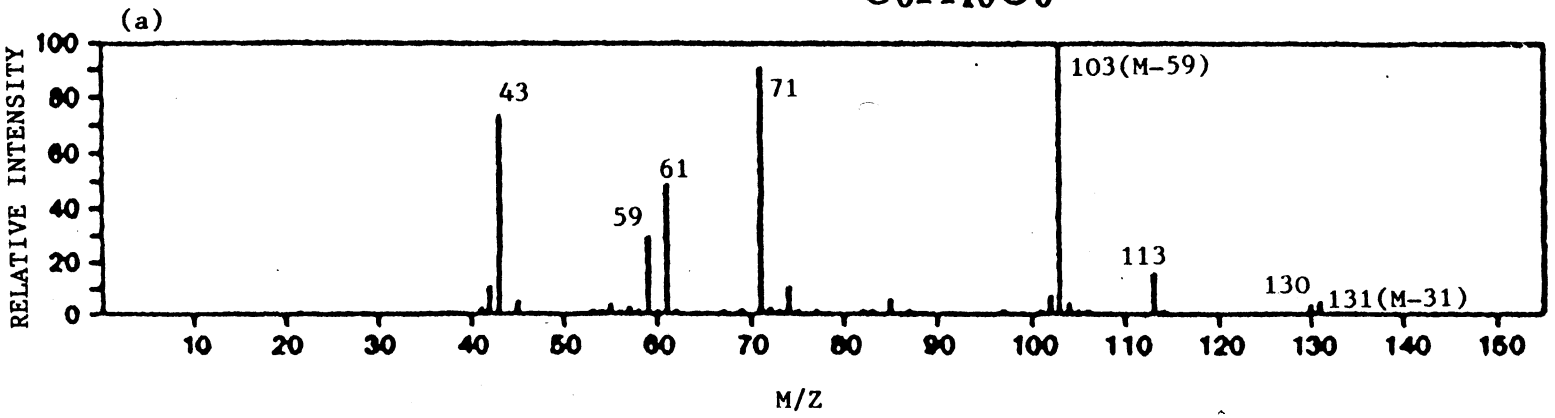


Figure 10. (a) Standard Mass Spectrum of Malic Acid, Dimethyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

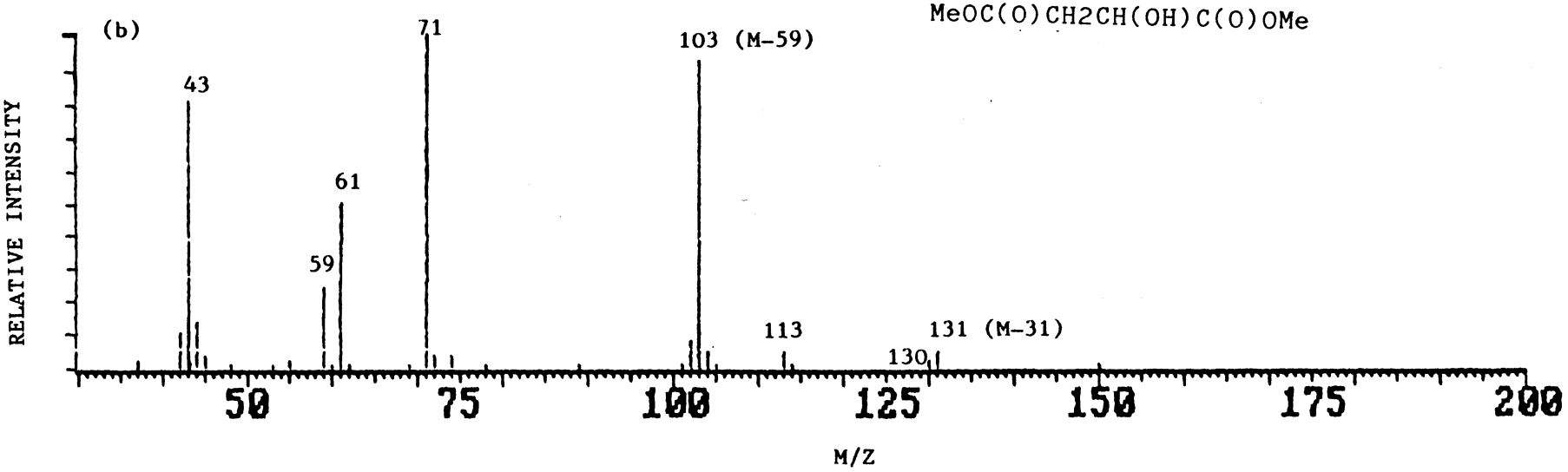
(b) Obtained Mass Spectrum of Malic Acid, Dimethyl Ester, Corresponding to Peak 12. (Sample: methylated methanol fraction of new wheat straw.)

Malic acid, dimethyl ester

$C_6H_{10}O_5$



MeOC(O)CH2CH(OH)C(O)OMe



Fumaric acid and malic acid do not exist in old wheat straw as indicated by two missing peaks (peak 8 and 12) in Fig. 6.

Shilling and his colleagues also isolated malic acid and succinic acid from aqueous extracts of rye (54). They indicated that malic acid was one of the responsible phytotoxins that inhibited hypocotyl and root growth of Chenopodium album and Amaranthus retroflexus, while succinic acid had no inhibitory effects. Among these four citric cycle acids, fumaric acid was most often reported as a possible allelopathic substance, inhibiting seedling growth of various weeds and crops (55, 56, 57). These citric cycle acids, however, are not likely to be the major allelochemicals in the inhibitory straw extracts. One study reported that no inhibitions were observed at concentrations up to 10^{-3}-10^{-2}M of these compounds on oat germination tests (58). In nature, they provide substracts for wheat plants and bacteria, the bacteria can then produce allelochemicals (59, 60).

Another dicarboxylic acid identified is nonanedioic acid (peak 26). The spectrum of this compound along with its standard spectrum are shown in Fig. 11. The compound has not been reported as an allelopathic substance. This compound was not detected in old wheat straw though there is a small peak near its retention time in Fig. 6.

Fig. 12 and Fig. 13 show the spectra of compounds presented by peaks 21 and 22. They were tentatively identified as two sugars, β-D-talofuranose, 1,2:5,6-bis-O-

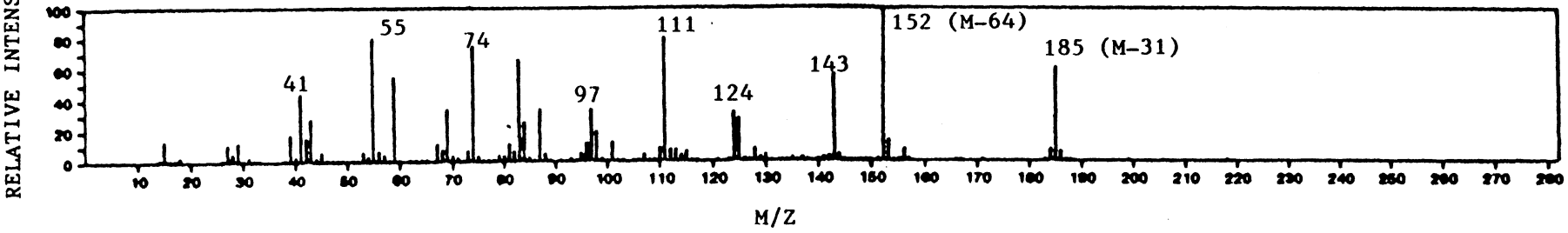
Figure 11. (a) Standard Mass Spectrum of Nonanedioic Acid, Dimethyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Nonanedioic Acid Dimethyl Ester, Corresponding to Peak 26. (Sample: methylated methanol fraction of new wheat straw.)

Nonanedioic acid, dimethyl ester

$C_{11}H_{20}O_4$

(a)



MeOC(O)(CH2)7C(O)OMe

(b)

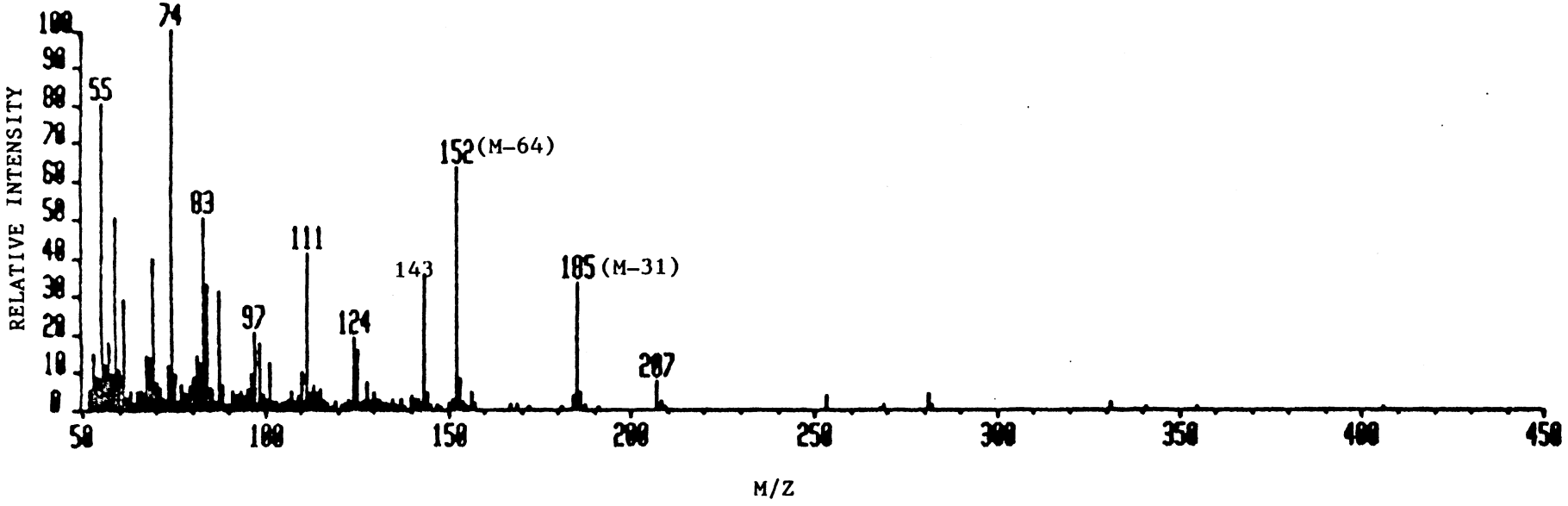
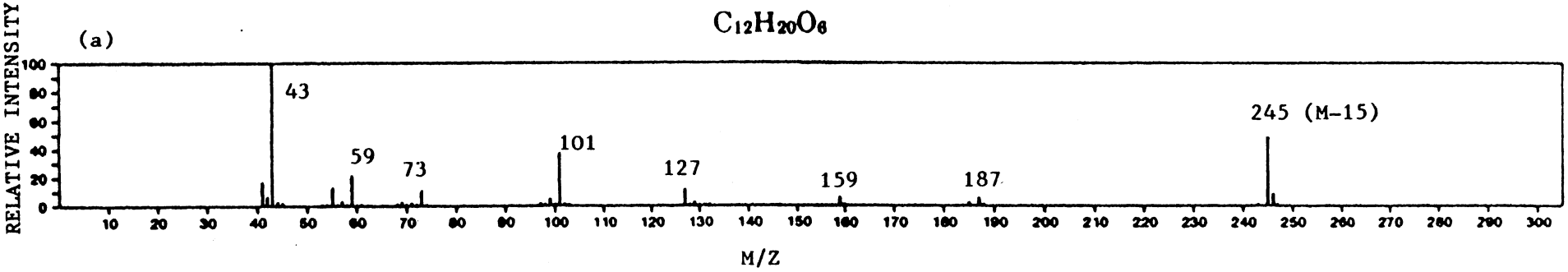


Figure 12. (a) Standard Mass Spectrum of β-D-Talofuranose, 1,2:5,6-bis-O-(1-methylethylidene). (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of β-D-Talofuranose, 1,2:5,6-bis-O-(1-methylethylidene), Corresponding to Peak 21. (Sample: methylated methanol fraction of new wheat straw.)

β-D-Talofuranose, 1,2:5,6-bis-*O*-(1-methylethylidene)

$C_{12}H_{20}O_6$



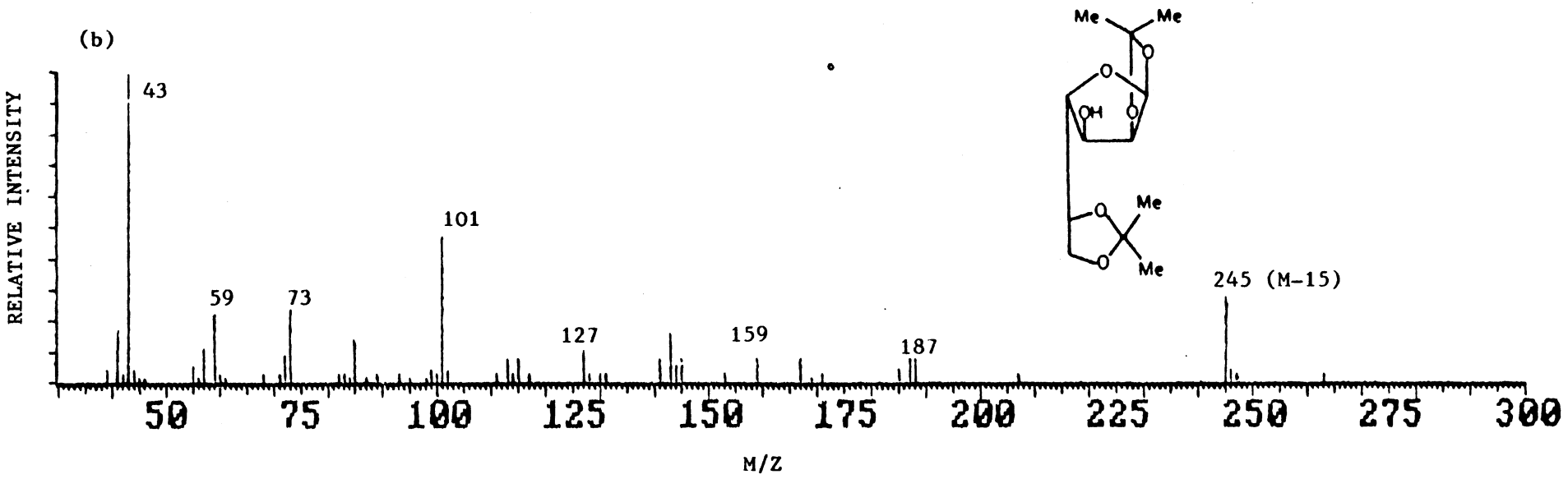
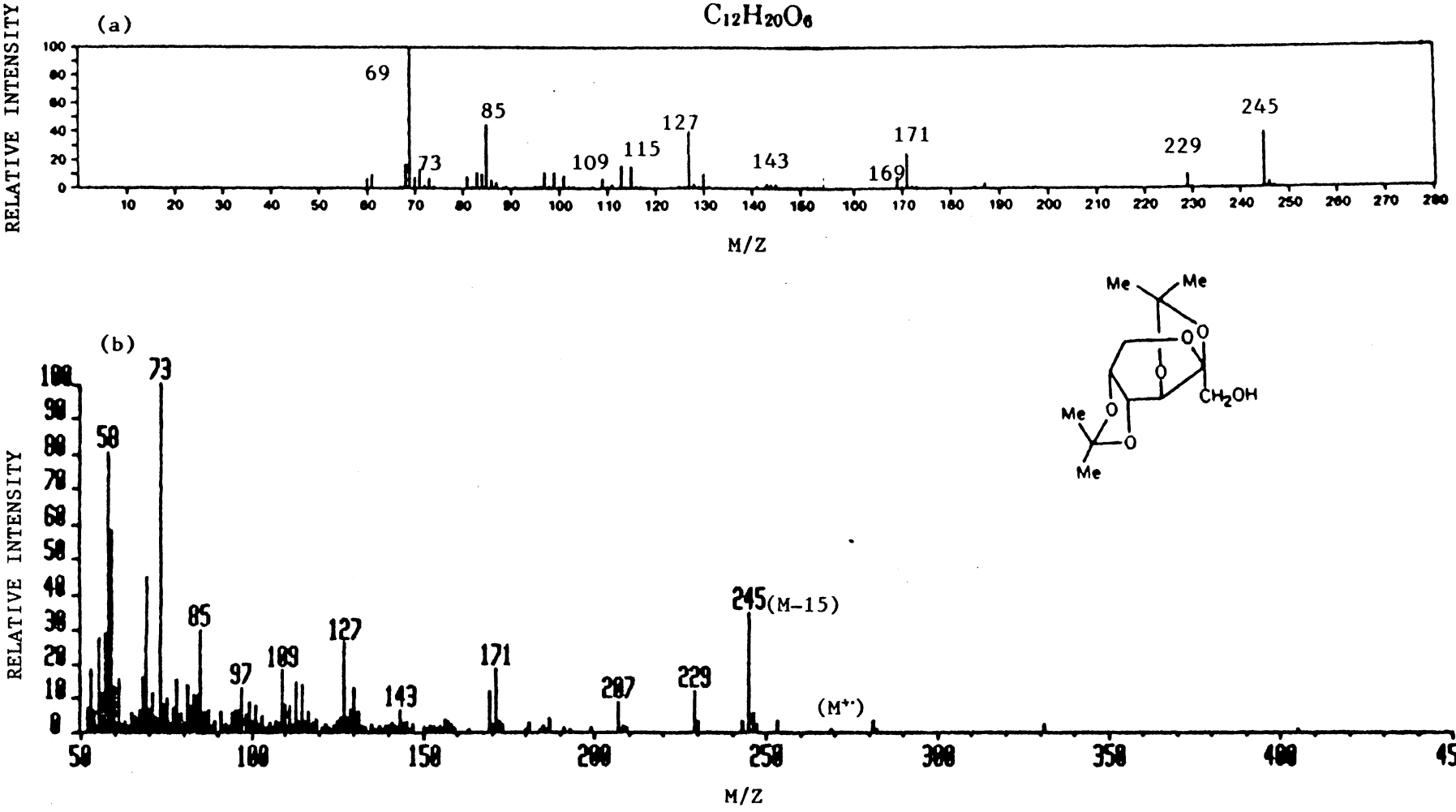


Figure 13. (a) Standard Mass Spectrum of β-D-Fructopyranose, 2,3:4:5-bis-O-(1-methylethylidene). (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of β-D-Fructopyranose, 2,3:4,5-bis-O-(1-methylethylidene), Corresponding to Peak 22. (Sample: methylated methanol fraction of old wheat straw.)

β-D-Fructopyranose, 2,3:4,5-bis-*O*-(1-methylethylidene)

$C_{12}H_{20}O_6$



(1-methylethyledene) and β-D-fructopyranose, 2,3:4,5-bis-O-(1-methylethylidene). The allelopathic activity of them is unknown. The identification of the sugars explains the stickiness of the crude aqueous extracts of wheat straw. β-D-fructopyranose, 2,3:4,5-bis-O-(1-methylethyledene) was found in old wheat straw only.

The highest peak (peak 28) in the spectra was identified as D-mannitol, 1,2:3,4:5,6-tris-O-(1-methylethylidene) (Fig. 14). No allelopathic effects of this compound have been reported.

Peak 36 and 39 were identified as methyl palmitate (Fig. 15) and methyl stearate (Fig. 16). Although some studies showed that they are allelochemicals (12, 37, 38), no inhibition were found by Cast (61) with either individual standard compounds or their various combinations, therefore they are not responsible for allelopathic effects in wheat straw and soil association.

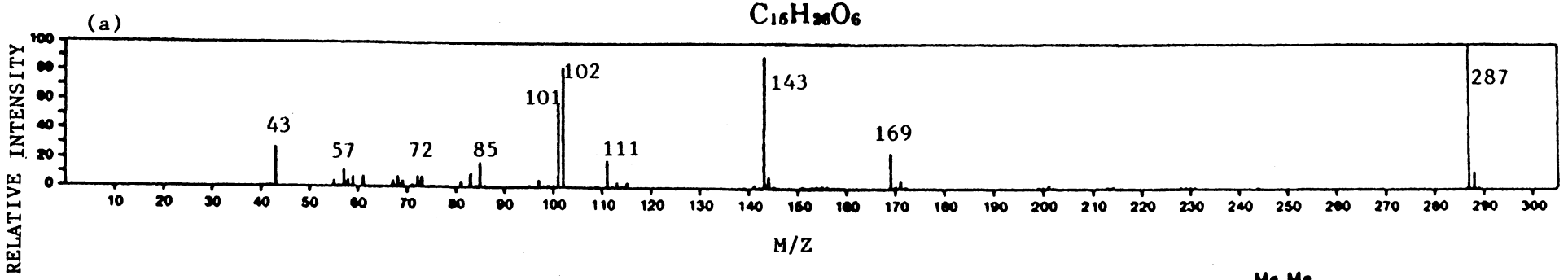
Fig. 17 and Fig. 18 are the spectra of two unsaturated long-chain fatty acid methyl esters, 11,14-eicosadienic acid, methyl ester and oleic acid, methyl ester, respectively. Both of them were found in new wheat straw (represented by peaks 37 and 38), whereas only 11,14-eicosadienic acid methyl ester (represented by peak 37) was found in old wheat straw. Peak 38 was missing. These two unsaturated long-chain fatty acids are more likely to be inhibitory in the bioassays than saturated fatty acids. Ibrahim et al (37) indicated in their work that the sodium salt of oleic acid was about 18% more inhibitory than that

Figure 14. (a) Standard Mass Spectrum of D-Mannitol, 1,2:3,4:5,6-tris-O-(1-methylethylidene). (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of D-Mannitol 1,2:3,4:5,6-tris-O-(1-methylethylidene), Corresponding to Peak 28. (Sample: methylated methanol fraction of new wheat straw.)

D-Mannitol, 1,2:3,4:5,6-tris-*O*-(1-methylethylidene)

$C_{15}H_{26}O_6$



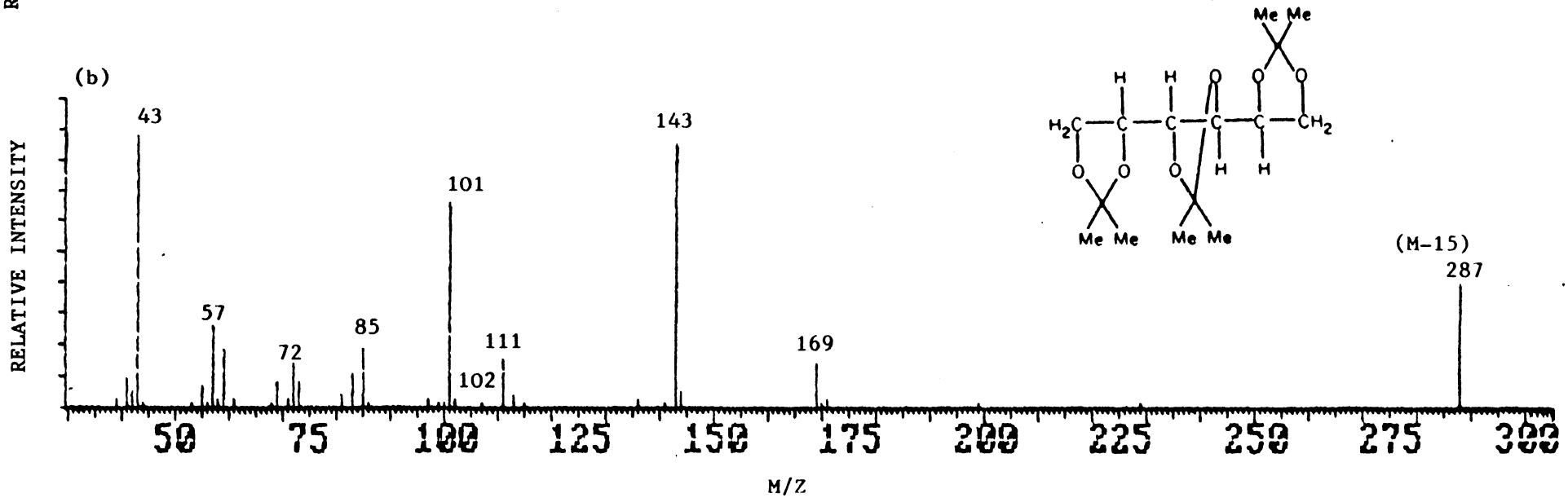


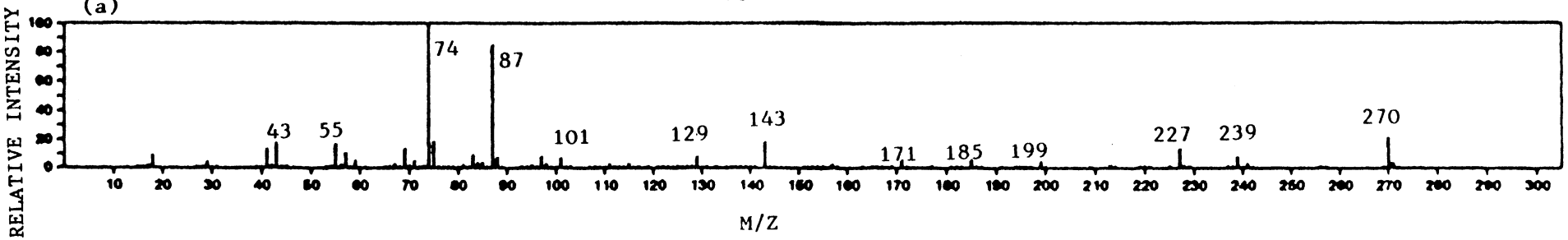
Figure 15. (a) Standard Mass Spectrum of Palmitate, Methyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Palmitate, Methyl Ester, Corresponding to Peak 36. (Sample: methylated methanol fraction of new wheat straw.)

Methyl palmitate

$C_{17}H_{34}O_2$

(a)



(b)

Me(CH2)14C(O)OMe

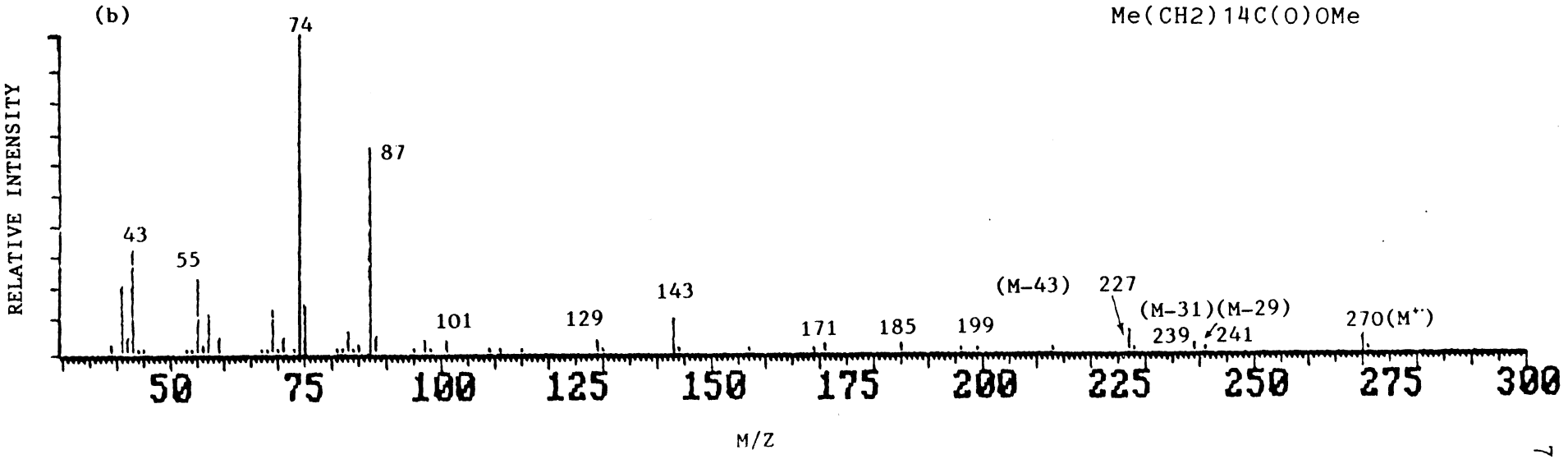
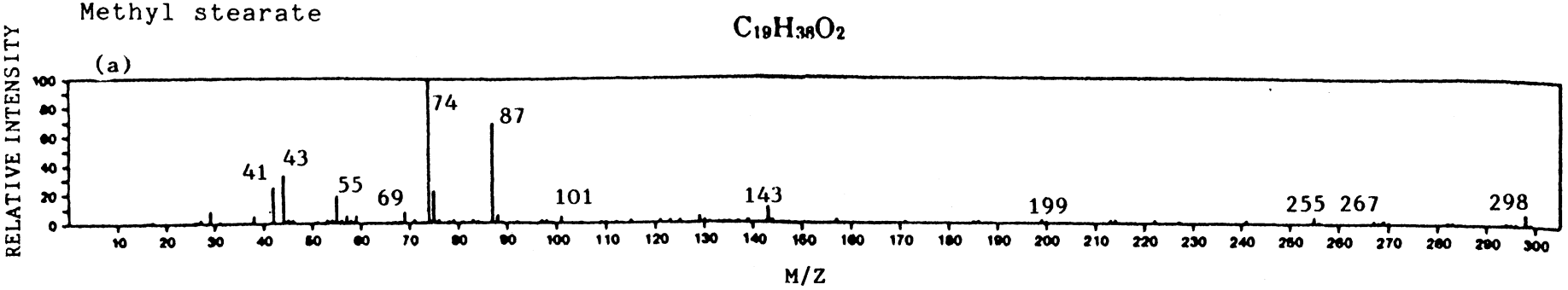


Figure 16. (a) Standard Mass Spectrum of Stearate, Methyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Stearate, Methyl Ester, Corresponding to Peak 39. (Sample: methylated methanol fractions of new wheat straw.)

Methyl stearate

$C_{19}H_{38}O_2$



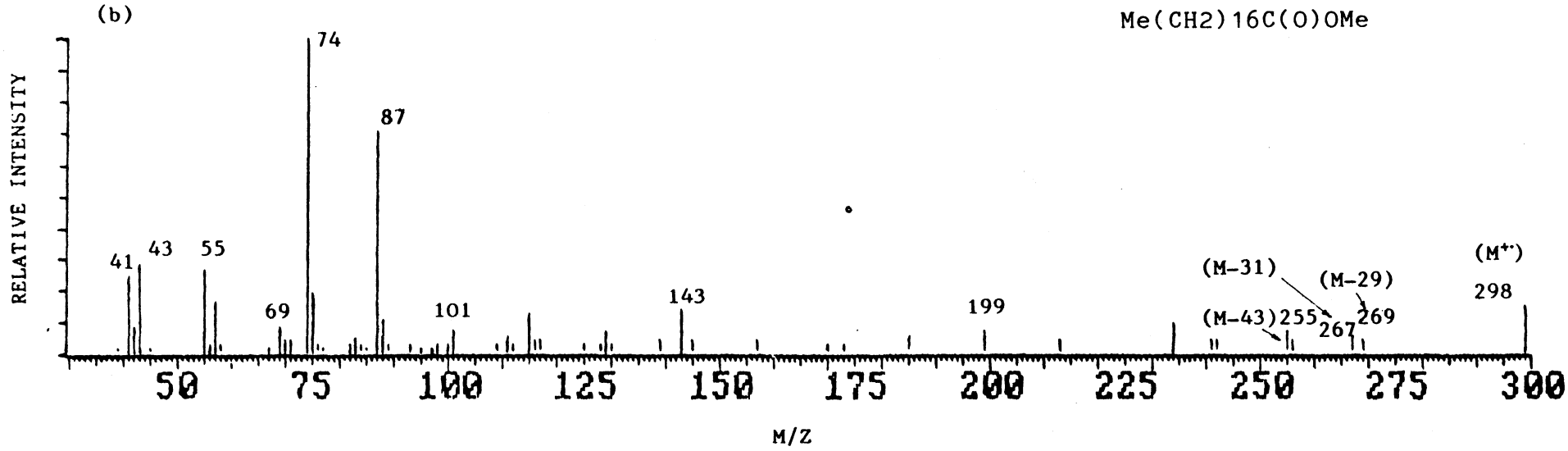
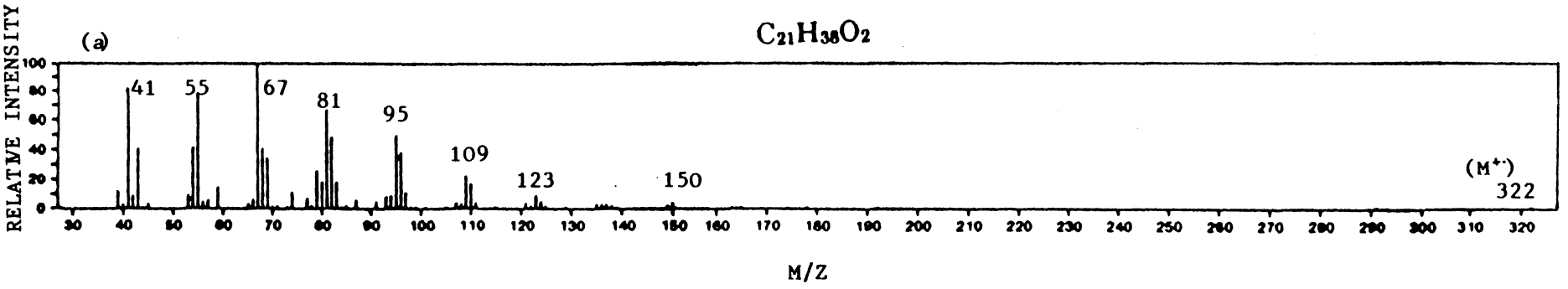


Figure 17. (a) Standard Mass Spectrum of 11,14-Eicosadienoic Acid, Methyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of 11,14-Eicosadienoic Acid, Methyl Ester, Corresponding to Peak 37. (Sample: methylated methanol fraction of new wheat straw.)

11,14-Eicosadienoic acid, methyl ester



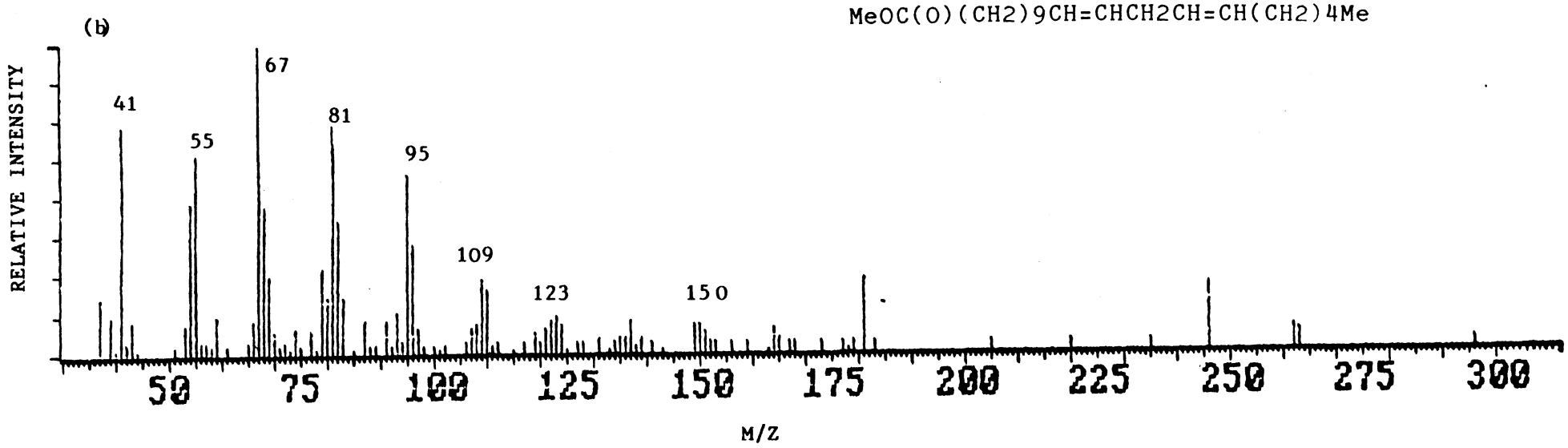


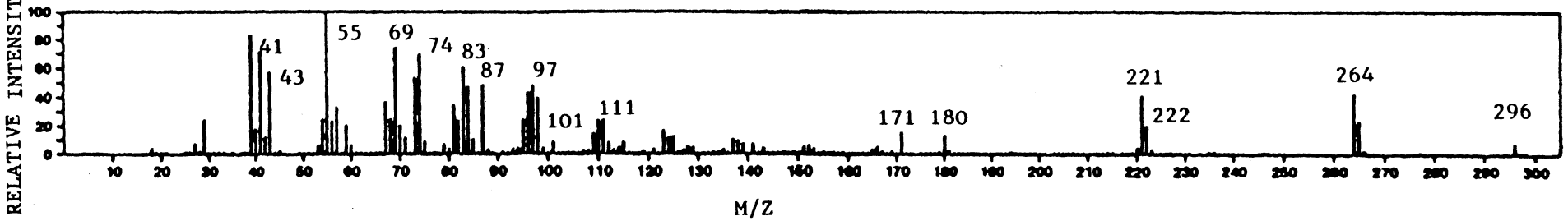
Figure 18. (a) Standard Mass Spectrum of Oleic Acid, Methyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Oleic Acid, Methyl Ester Corresponding to Peak 38. (Sample: methylated methanol fraction of new wheat straw.)

Methyl oleate

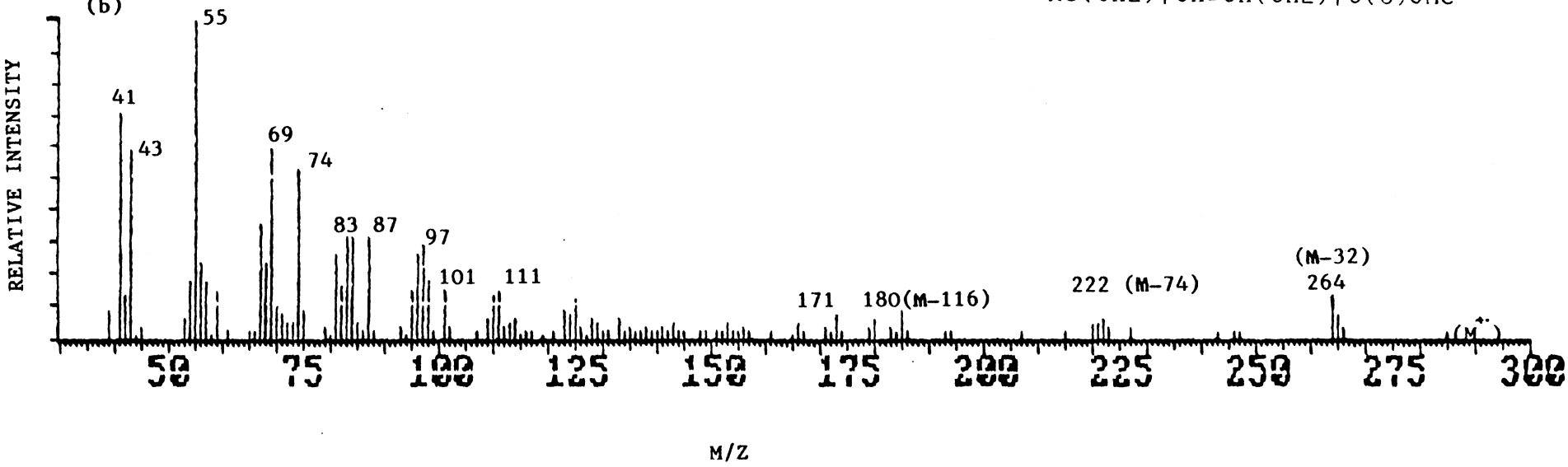
(a)

$C_{19}H_{36}O_2$



Me(CH2)7CH=CH(CH2)7C(O)OMe

(b)



of 11,14-eicosodienic acid against seed germination and seedling growth of bermudagrass at 50ppm level. The oleic acid containing fraction of new wheat straw extract also showed about 10% more inhibition than that of old wheat straw extract (Table V line 3 and Table VI, line 3), in which no oleic acid but 11,14-eicosodienoic acid was found.

Based on the bioassay results (Table V, line 4 and 5), the chemical nature of CH_2Cl_2 fraction and $CHCl_3$ fraction of new wheat straw was very interesting. The reconstructed total ion current chromatograms of these two fractions were shown in Fig. 19 and Fig 20, respectively. The major components isolated in these two fractions were various types of phthalate plasticizers. Two most common types of plasticizers, 1,4-benzendicarboxylic acid, dimethyl ester (peak at scan 608) and 1,2-benzendicarboxylic acid, bis-(2-methylethyl) ester (peak at scan 976), are presented in Fig. 21 and Fig. 22, respectively. Plasticizers were found elsewhere in almost every extract, but they were centralized mainly in the CH_2Cl_2 and $CHCl_3$ Fractions. The total quantity of these compounds are relatively high. They were often considered as laboratory contaminants from various plastic-made wares; however, this interpretation is not believed to be correct in this study. All-glass equipment, Teflon stopcocks and centrifuge tubes were used throughout the experimental procedures. Thus, plasticizers are actual components extracted from wheat straw and soil. They may be naturally occurring compounds or, otherwise, introduced into the natural environment from external sources.

Figure 19. Reconstructed Total Ion Current of Chromatogram of CH_2Cl_2 Fraction of New Wheat Straw. (Peaks labeled represent the compounds identified and/or discussed.)

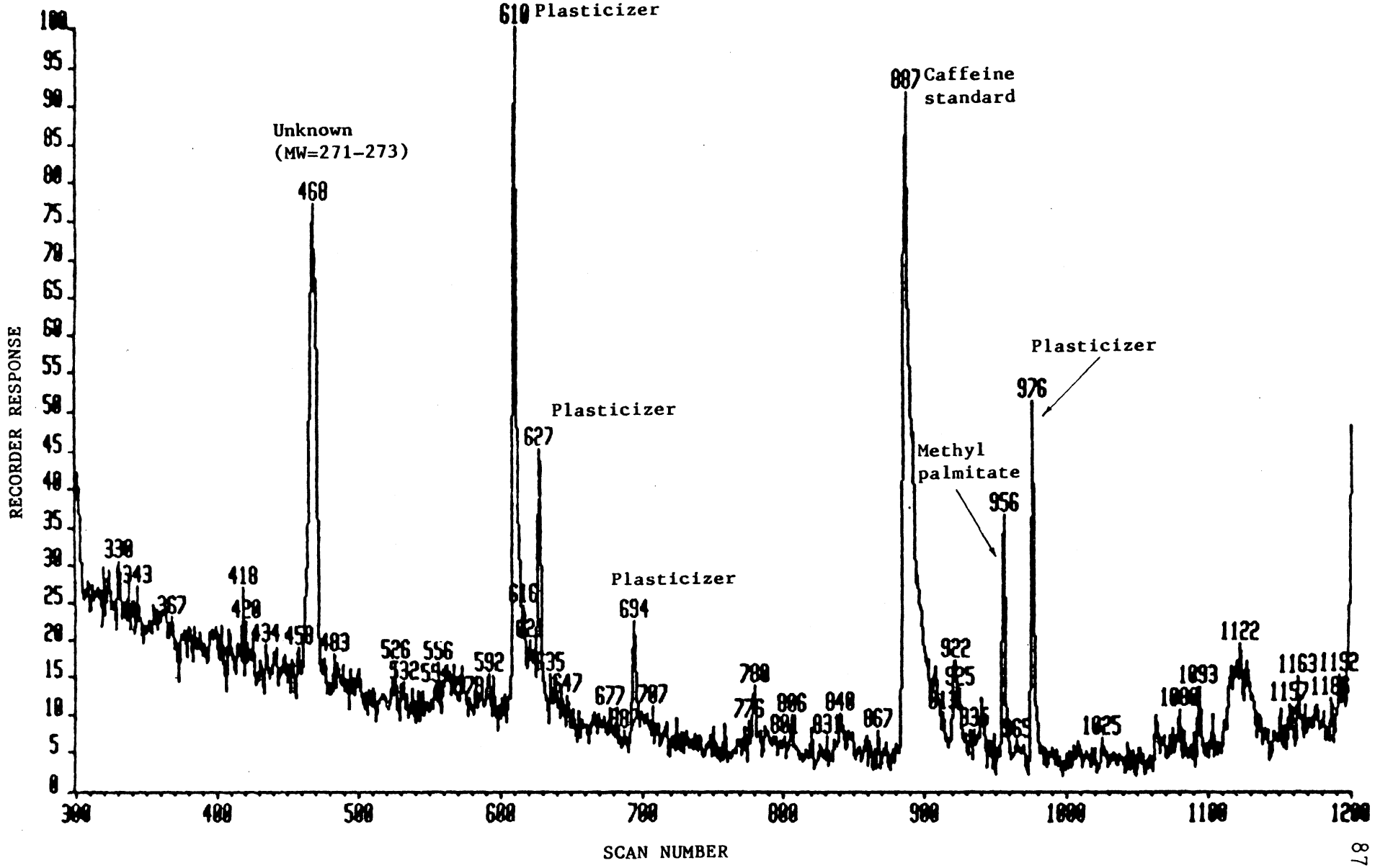

Plasticizer
Caffeine standard
Unknown (MW=271-273)
Plasticizer
Plasticizer
Methyl palmitate
Plasticizer
RECORDER RESPONSE
SCAN NUMBER

Figure 20. Reconstructed Total Ion Current Chromatogram of $CHCl_3$ Fraction of New Wheat Straw. (Peaks labeled represent the compounds identified and/or discussed.

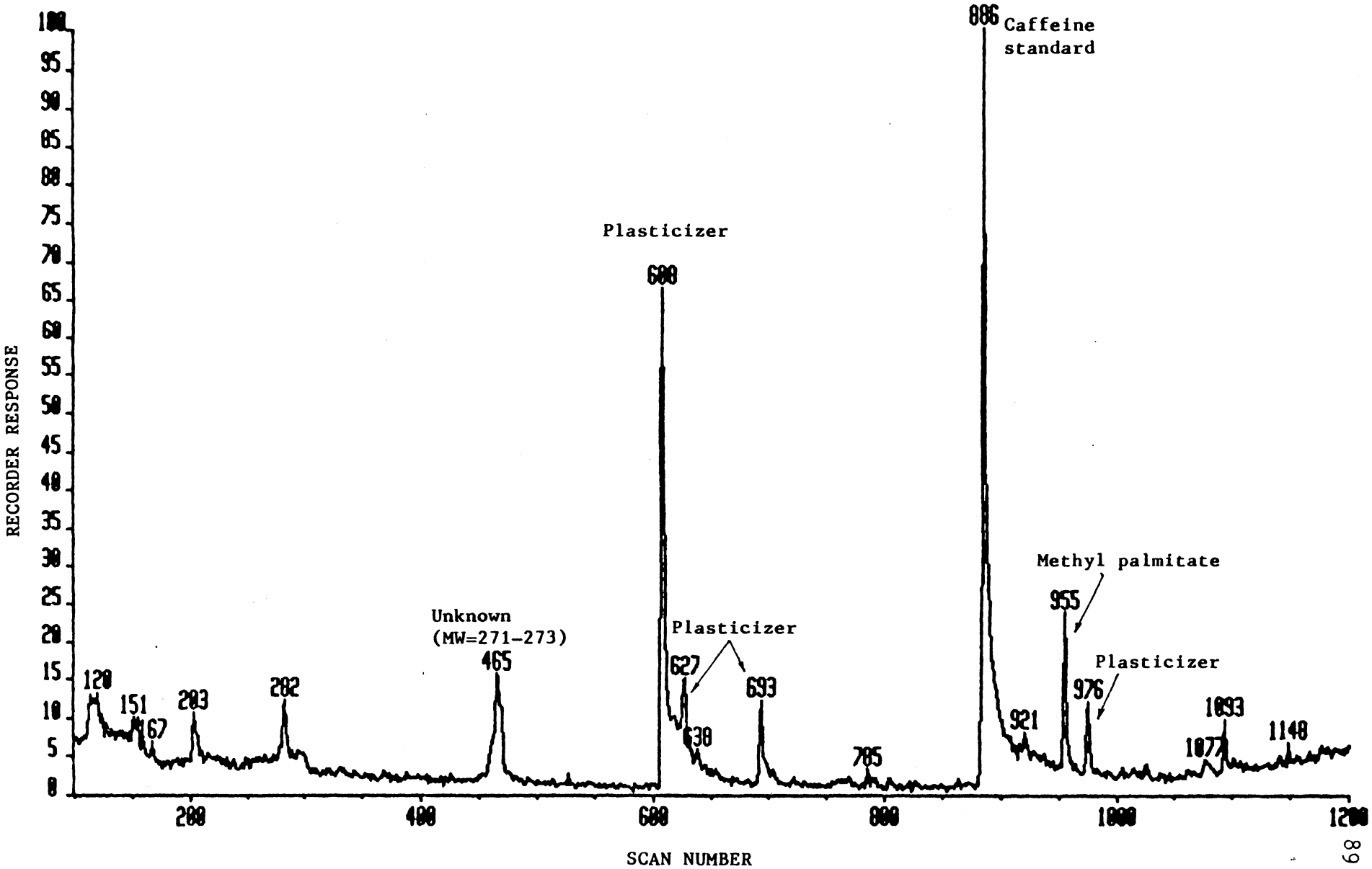

RECORDER RESPONSE
SCAN NUMBER
886
Caffeine
standard
Plasticizer
600
Unknown
(MW=271-273)
465
Plasticizer
627
693
Methyl palmitate
955
Plasticizer
976
120
151
167
203
282
630
705
921
1077
1093
1140
200
400
600
800
1000
1200

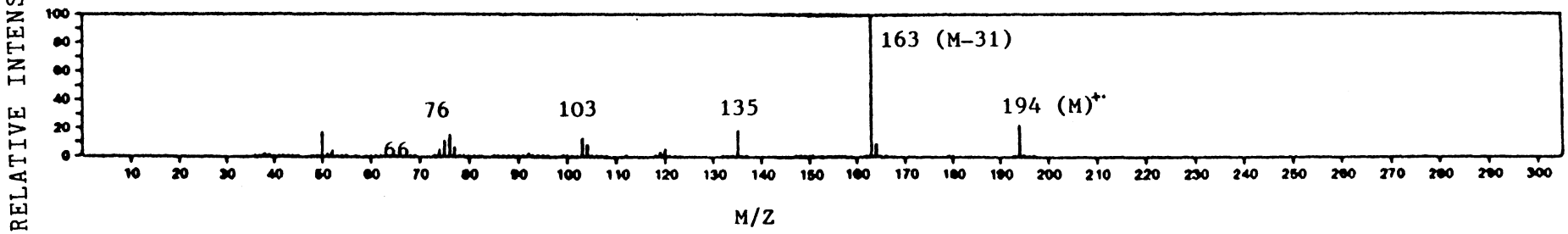
Figure 21. (a) Standard Mass Spectrum of 1,4-Benzenedicarboxylic Acid, Dimethyl Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of 1,4-Benzenedicarboxylic Acid, Dimethyl Ester, Corresponding to Peak at Scan 608. (Sample: CH_2Cl_2 fraction of new wheat straw.)

1,4-Benzenedicarboxylic acid, dimethyl ester

(a)

$C_{10}H_{10}O_4$



(b)

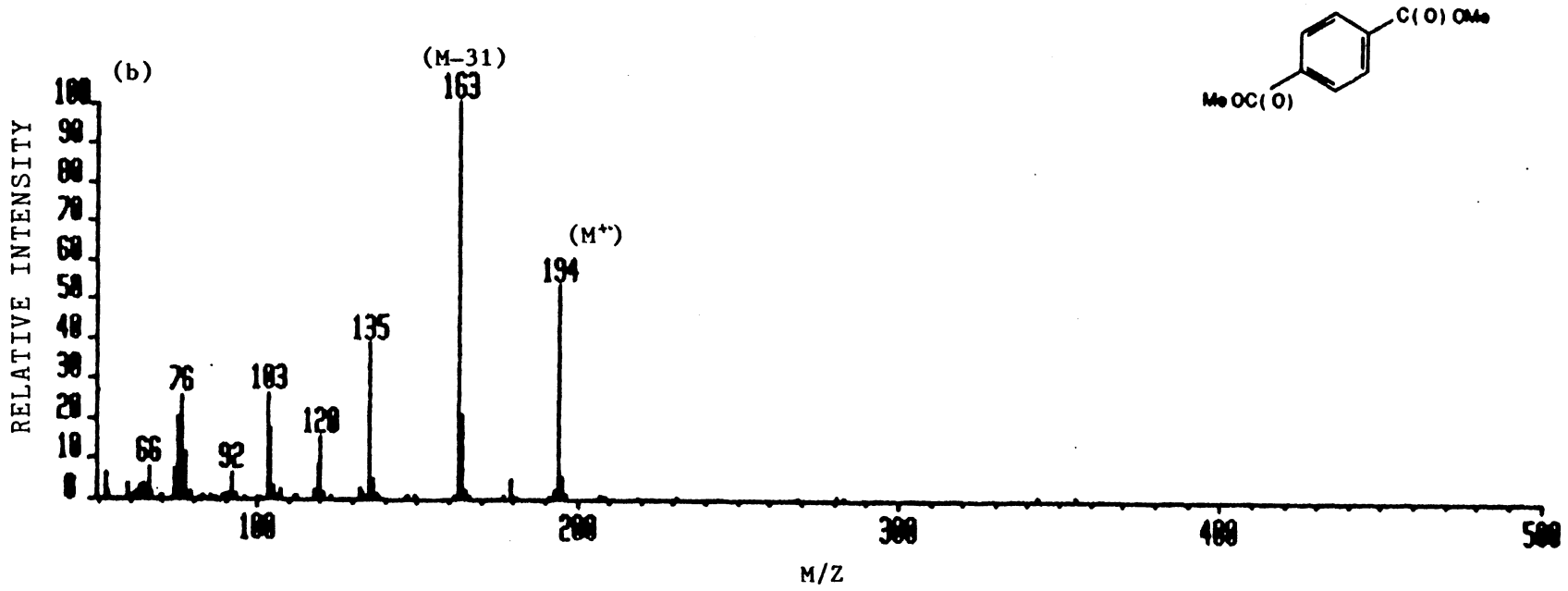
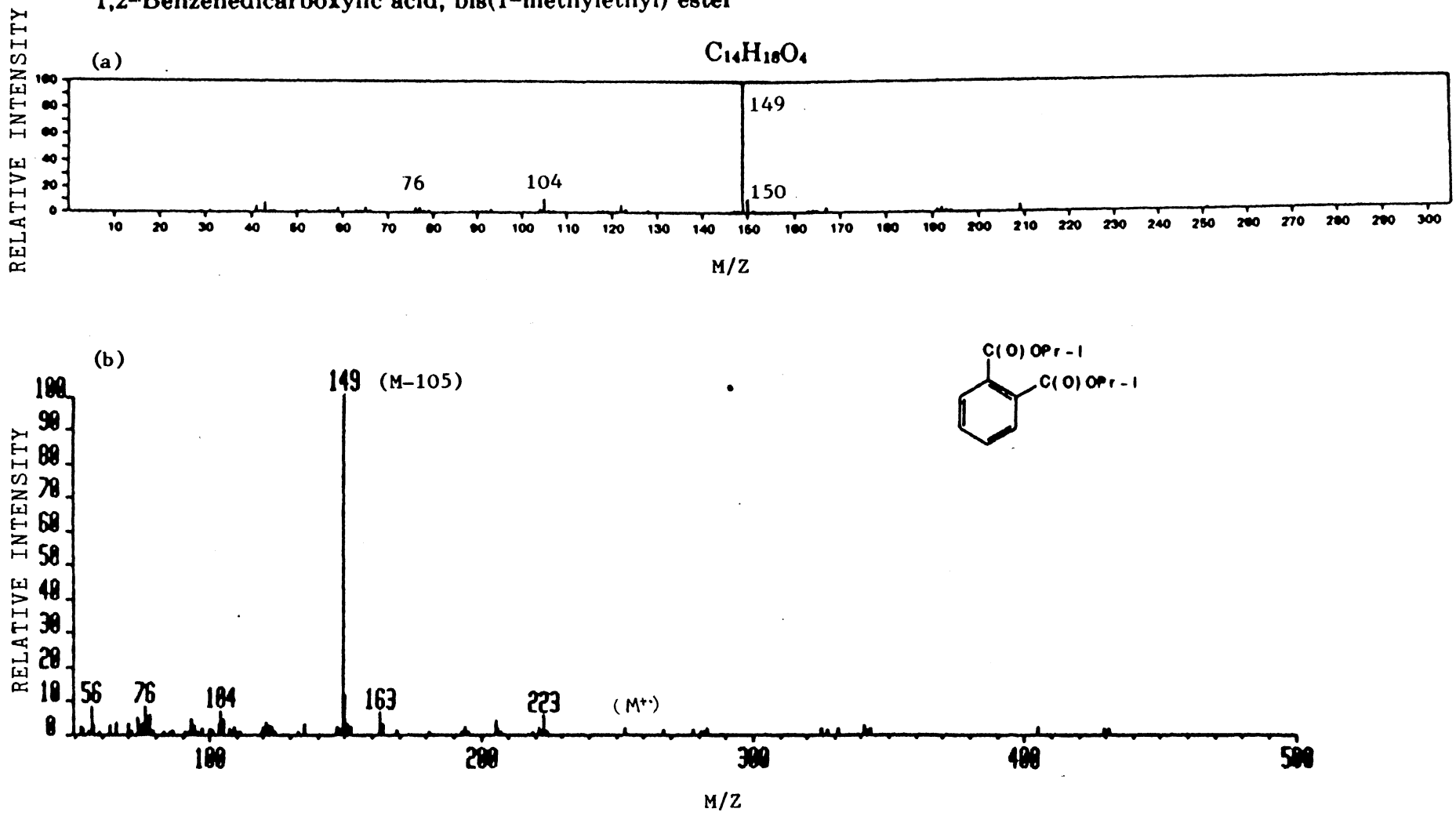


Figure 22. (a) Standard Mass Spectrum of 1,2-Benzenedicarboxylic Acid, Bis(1-methylethyl) Ester. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of 1,2-Benzenedicarboxylic Acid, Bis(1-methylethyl) Ester, Corresponding to Peak at Scan 976. (Sample: CH_2Cl_2 fraction of new wheat straw.)

1,2-Benzenedicarboxylic acid, bis(1-methylethyl) ester



both the CH_2Cl_2 and $CHCl_3$ fractions of new straw (represented by peak at scan 956 in Fig. 19 and at scan 955 in Fig. 20). Its spectrum obtained is identical to the one showed in Fig. 15.

Figure 23 shows the spectrum of an unknown compound obtained mainly in the CH_2Cl_2 fraction (peak at scan 468 in Fig. 19). A small peak that represents this compound also appeared in the $CHCl_3$ fraction (peak at scan 465 in Fig. 20). An effort was made on determining the structure of this unknown compound which appeared very toxic to the growth of wheat seedlings (32% inhibition, Table VI), assuming the elimination of all the other components as allelochemicals discussed above is correct. Due to the lack of a high-resolution instrument for identification, and the delay in obtaining results from VG Analytical Instruments in England, this compound still remains unidentified. The possible molecular weight of 271-273 was a conjecture based on the obtained mass spectral data.

Fig. 24 and Fig. 25 show the reconstructed total ion current chromatograms of CH_2Cl_2 fraction of old wheat straw and $CHCl_3$ fraction of old wheat straw, respectively. Contrary to those of new wheat straw, no chemical compounds were found except for the peaks which corresponded with caffeine and a few of plasticizers. This result agreed with the lack of activities of these two fractions examined in bioassays (Table V, line 5 and 6).

Figure 26 and Figure 27 are the reconstructed total ion

Figure 23. Obtained Mass Spectrum of an Unknown Compound, Corresponding to Peak at Scan 468. (Sample: CH_2Cl_2 fraction of New Wheat Straw.)

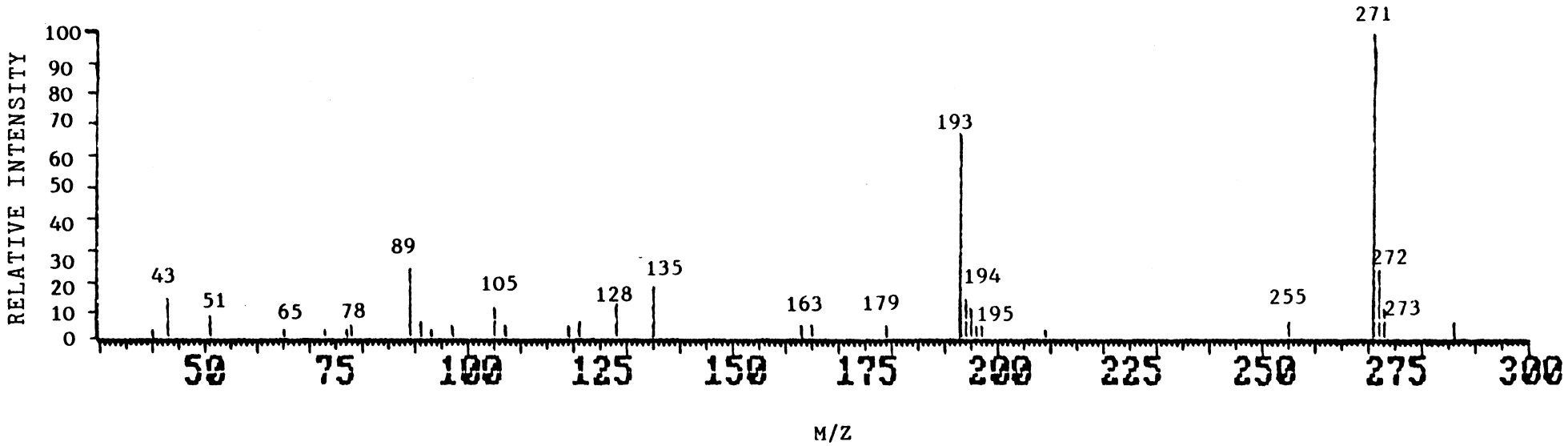

RELATIVE INTENSITY
100
90
80
70
60
50
40
30
20
10
0
43
51
65
78
89
105
128
135
163
179
193
194
195
255
271
272
273
50
75
100
125
150
175
200
225
250
275
300
M/Z

Figure 24. Reconstructed Total Ion Current Chromatogram of CH_2Cl_2 Fraction of Old Wheat Straw. (Peaks labeled represent the compounds identified and/or discussed.)

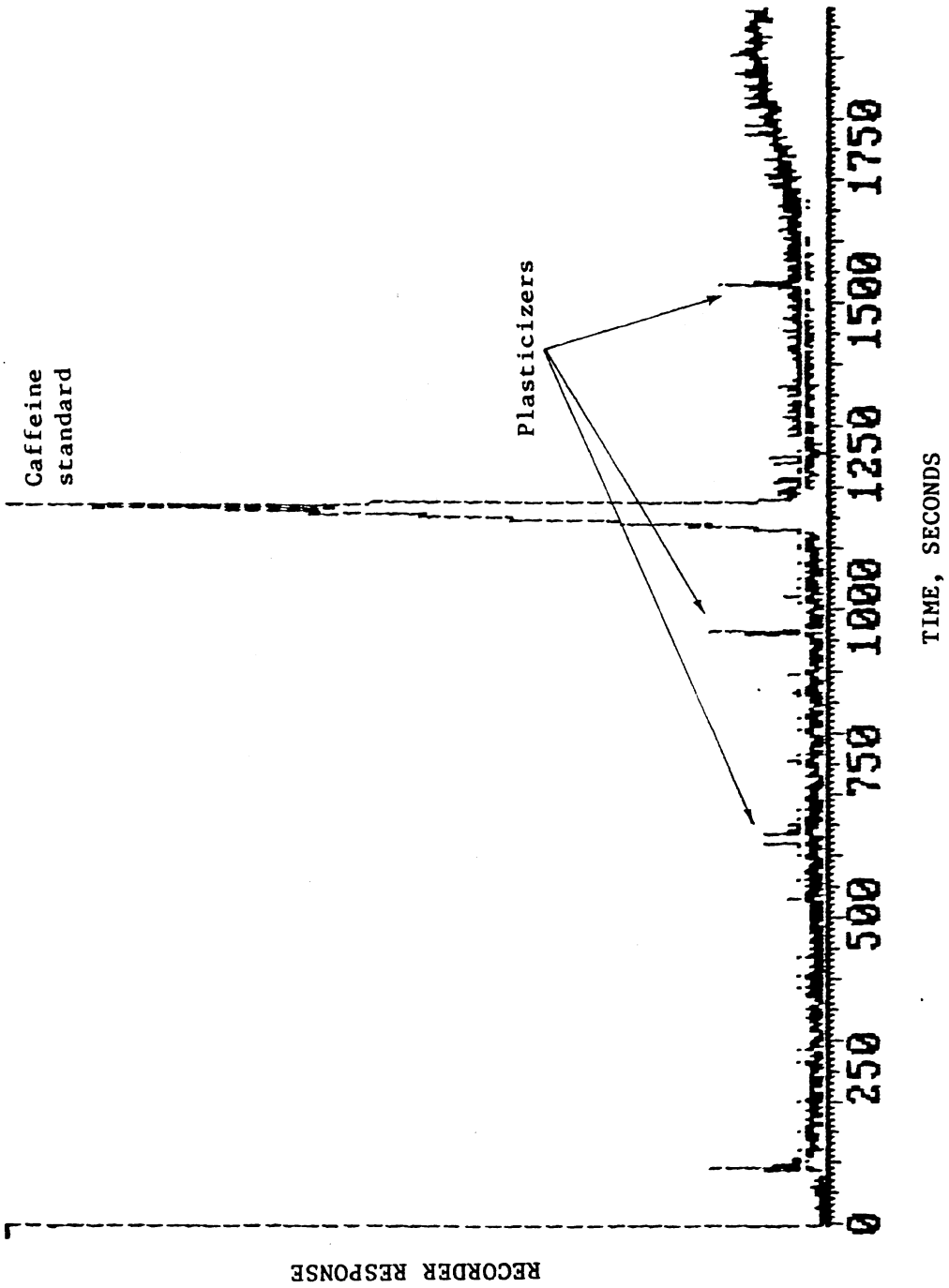

Caffeine
standard
Plasticizers
0
250
500
750
1000
1250
1500
1750
TIME, SECONDS
RECORDER RESPONSE

Figure 25. Reconstructed Total Ion Chromatogram of CHC_13 Fraction of Old Wheat Straw. (Peaks labeled represent the compounds identified and/or discussed.)

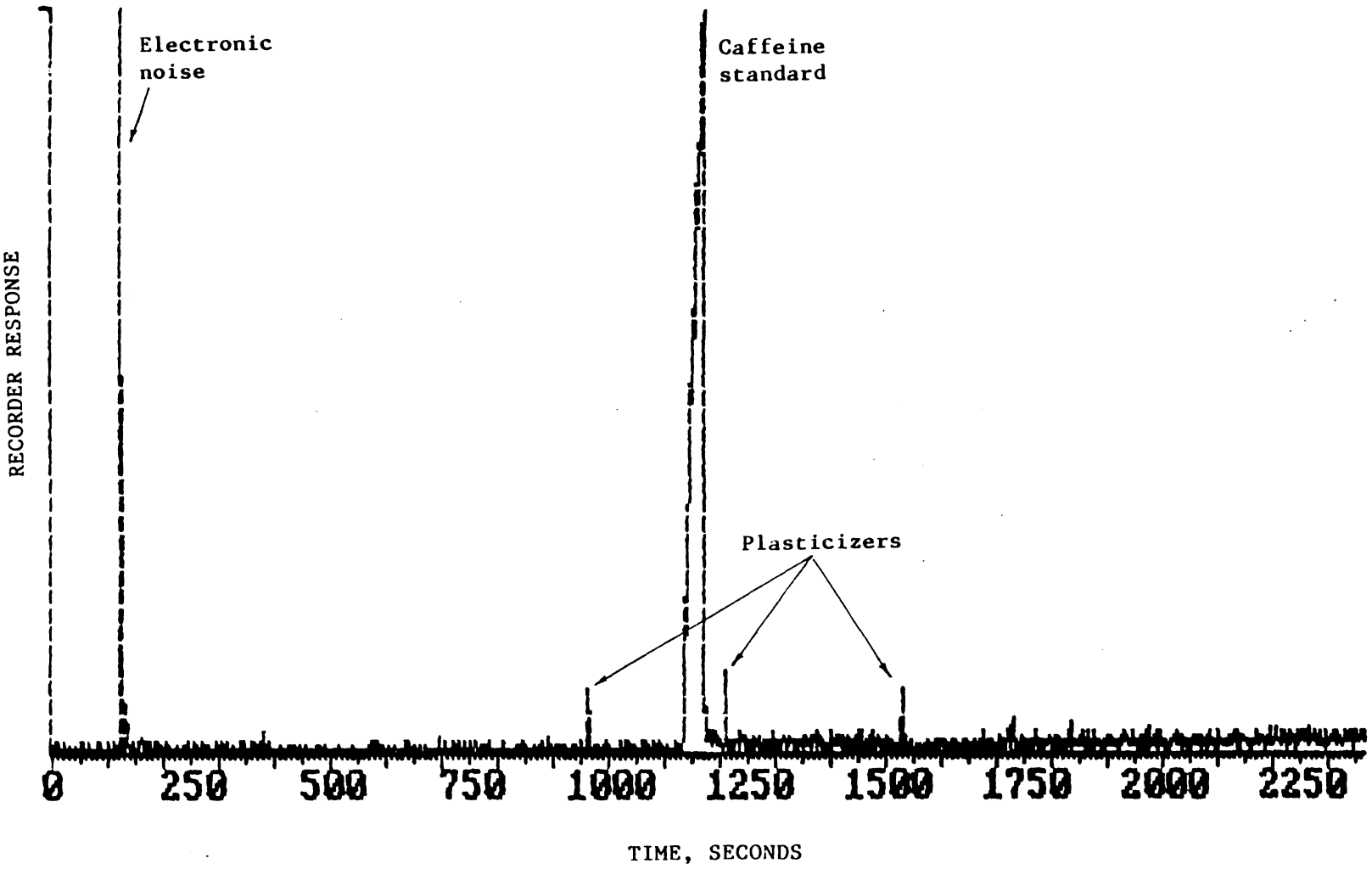

Electronic
noise
Caffeine
standard
Plasticizers
RECORDER RESPONSE
0
250
500
750
1000
1250
1500
1750
2000
2250
TIME, SECONDS

Figure 26. Reconstructed Total Ion Current Chromatogram of Methylated Methanol Fraction of CT Soil, June 1985. (Peaks labeled represent the compounds identified and/or discussed.)

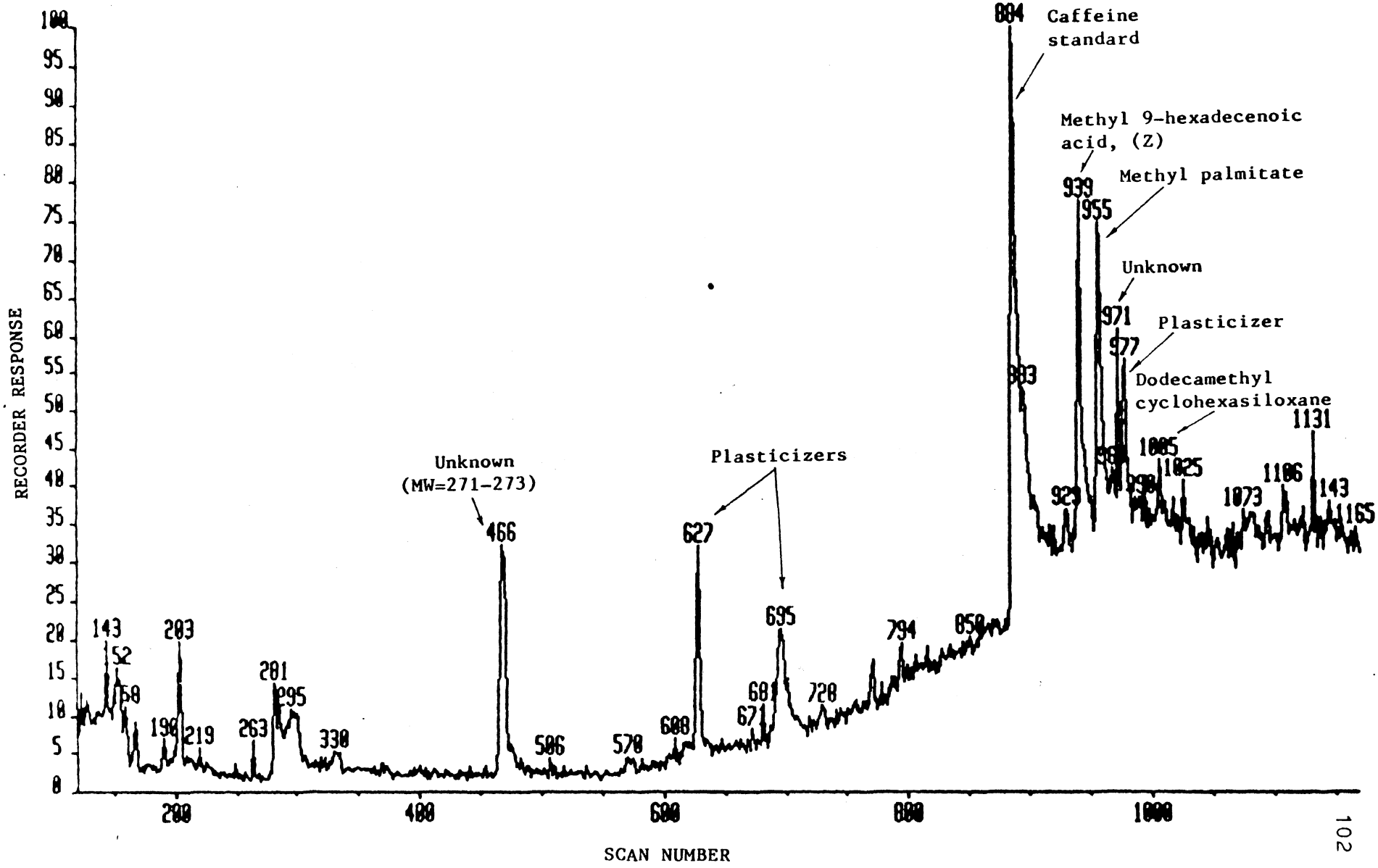
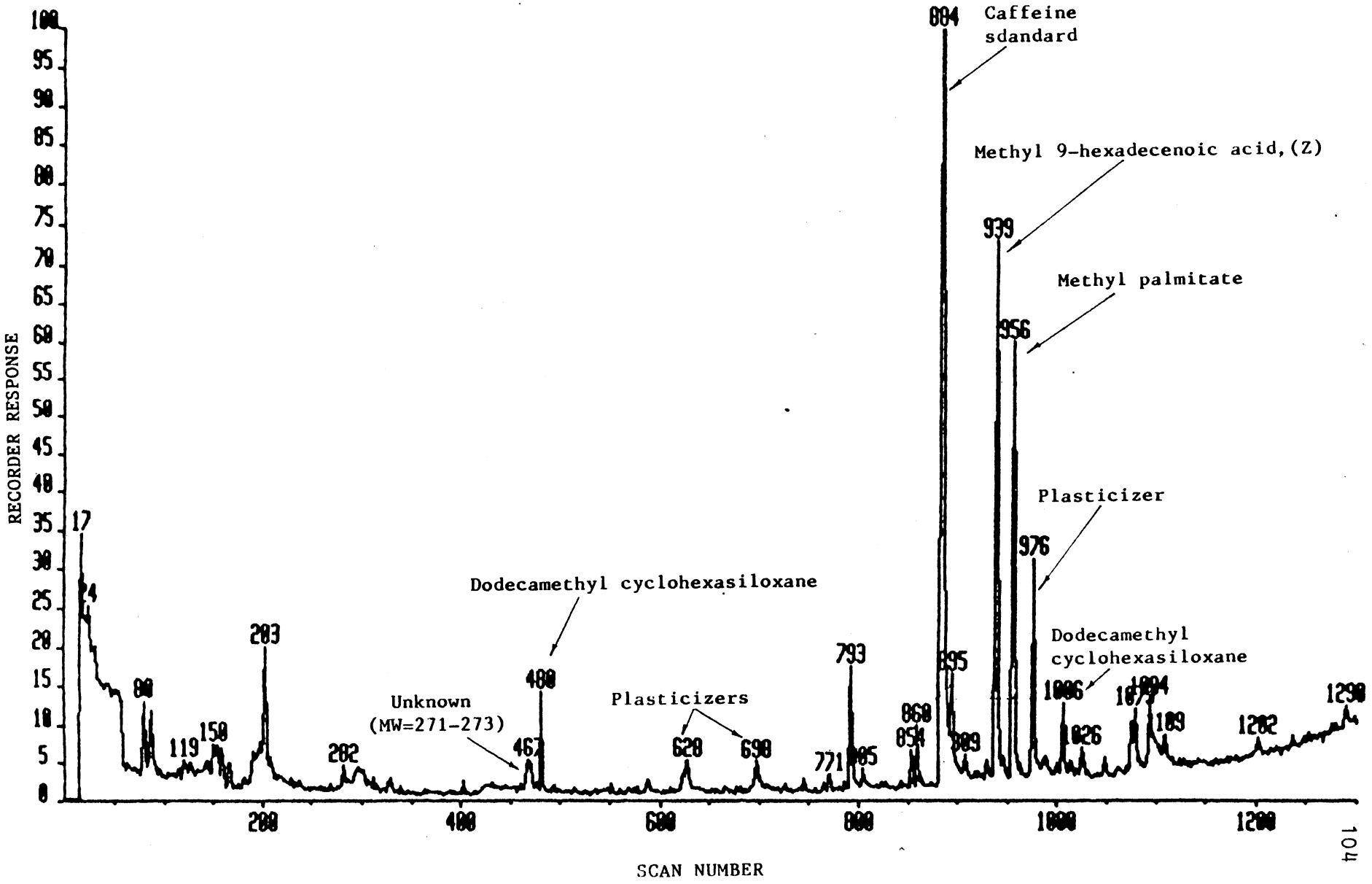

RECORDER RESPONSE
SCAN NUMBER
Caffeine standard
Methyl 9-hexadecenoic acid, (Z)
Methyl palmitate
Unknown
Plasticizer
Dodecamethyl cyclohexasiloxane
Unknown (MW=271-273)
Plasticizers
884
939
955
971
977
883
1131
1085
1025
1106
1073
143
165
203
281
295
466
627
695
794
858
728
681
671
608
570
506
330
263
219
196
52
50

Figure 27. Reconstructed Total Ion Current Chromatogram of Methylated Methanol Fraction of NT Soil, June 1985. (Peaks labeled represent the compounds identified and/or discussed.)


RECORDER RESPONSE
SCAN NUMBER
Caffeine
sdandard
Methyl 9-hexadecenoic acid,(Z)
Methyl palmitate
Plasticizer
Dodecamethyl cyclohexasiloxane
Dodecamethyl
cyclohexasiloxane
Unknown
(MW=271-273)
Plasticizers
884
939
956
976
793
283
488
462
628
698
771
17
24
80
119
150
202
895
869
854
860
1026
1189
1282
1298
200
400
600
800
1000
1200

current chromatograms of methylated CH_3OH fraction of basic extract of conventional-tillage soil and that of no-tillage soil. Corresponding peaks in these two chromatograms can be seen. Most of the components are the same in both soil extracts except for those represented by peaks at scan 466, scan 480 and scan 971 in Figures 26 and 27.

Based on the retention times and the mass spectral data obtained, most peaks were identified as labeled on Figures 26 and 27. Peak at scan 884 in both chromatograms represented the caffeine internal standard peak. Corresponding plasticizer peaks are also indicated on the chromatograms; the spectra of two of their major components were very similar to those shown in Fig. 21 and Fig. 22.

The mass spectrum of the peak at scan 466 in Fig.26 indicated that it represents the same unknown compound found in CH_2Cl_2 and $CHCl_3$ fractions of new wheat straw. The obtained spectrum of this compound in the CT soil extract is very similar to the spectrum presented in Fig. 23 (the spectra of the same compounds found in different extracts are not presented again in order to avoid repetition). A trace amount of this unknown in the no-tillage soil extract is indicated by a small peak at scan 467 in Figure 27. The corresponding bioassay results show that the methanol fraction of basic CT soil extract is 12% more inhibitory than that of NT soil extract (Table VII-B, line 7 and VIII-B, line 10).

Methyl palmitate (scan 935), methyl stearate (scan 1094) were found in both soil extracts. Their spectra are

identical to the spectra of these two compounds identified in straw extracts, and can be referred in Fig. 15 and Fig. 16, respectively. The low biological activities of these two acids tested on wheat seedling growth in terms of allelopathy have been discussed previously.

A methyl ester of another unsaturated fatty acid was found in both soil extracts. It was represented by peak at scan 939 in Figures 26 and 27, and was identified as 9-hexadecanoic acid, methyl ester. Figure 28 shows the obtained spectrum of this compound along with its standard spectrum. It has not been determined whether it is phytotoxic.

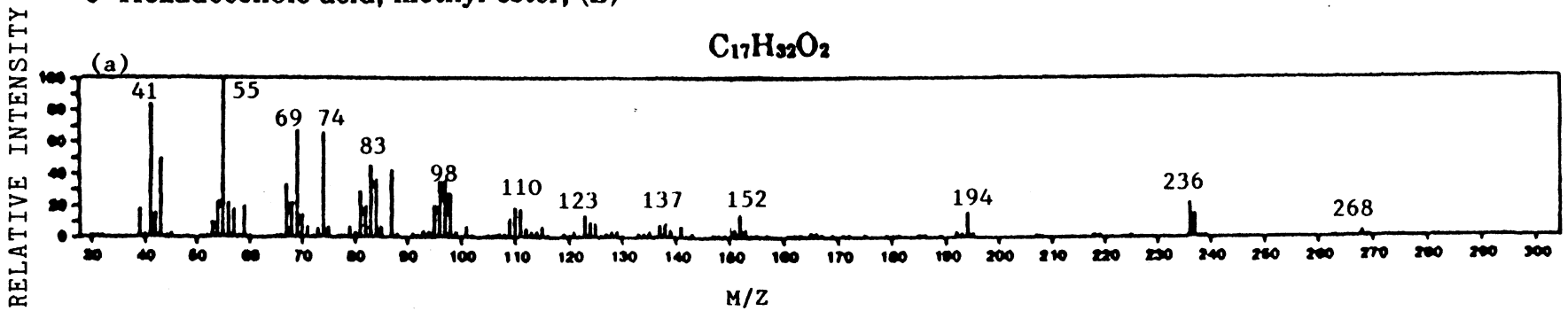
Peaks at scan 480 and scan 1005-1006 were identified as cyclohexasiloxane, dodecamethyl (Figure 29). It is probably the column coating material, which bled during the runs and spread throughout the column, with the concentration increasing as the temperature increased.

The chemical identification by gas chromatography/mass spectrometry is summarized in Table IX. The data presented in the table are based on the mass spectral data analysis discussed above.

Figure 28. (a) Standard Mass Spectrum of 9-Hexadecenoic Acid, Methyl Ester, (Z). (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of 9-Hexadecenoic Acid, Methyl Ester, (Z), Corresponding to Peak at Scan 939. (Sample: methylated methanol fractions of CT Soil, June 1985.)

9-Hexadecenoic acid, methyl ester, (*Z*)-



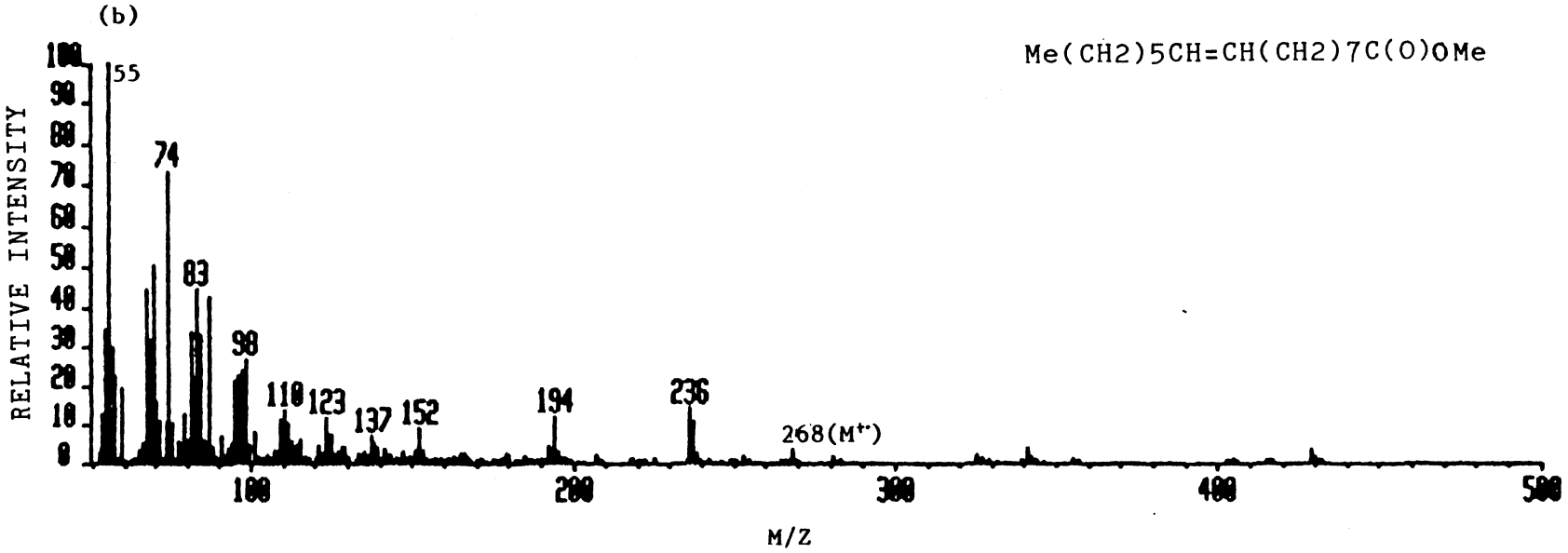


Figure 29. (a) Standard Mass Spectrum of Cyclohexasiloxane, Dodecamethyl. (Source: EPA/NIH Mass Spectral Data Base.)

(b) Obtained Mass Spectrum of Cyclohexasiloxane, Dodecamethyl, Corresponding to Peak at Scan 480. (Sample: methylated methanol fractions of NT soil, June 1985.)

Cyclohexasiloxane, dodecamethyl-

$C_{12}H_{36}O_6Si_6$

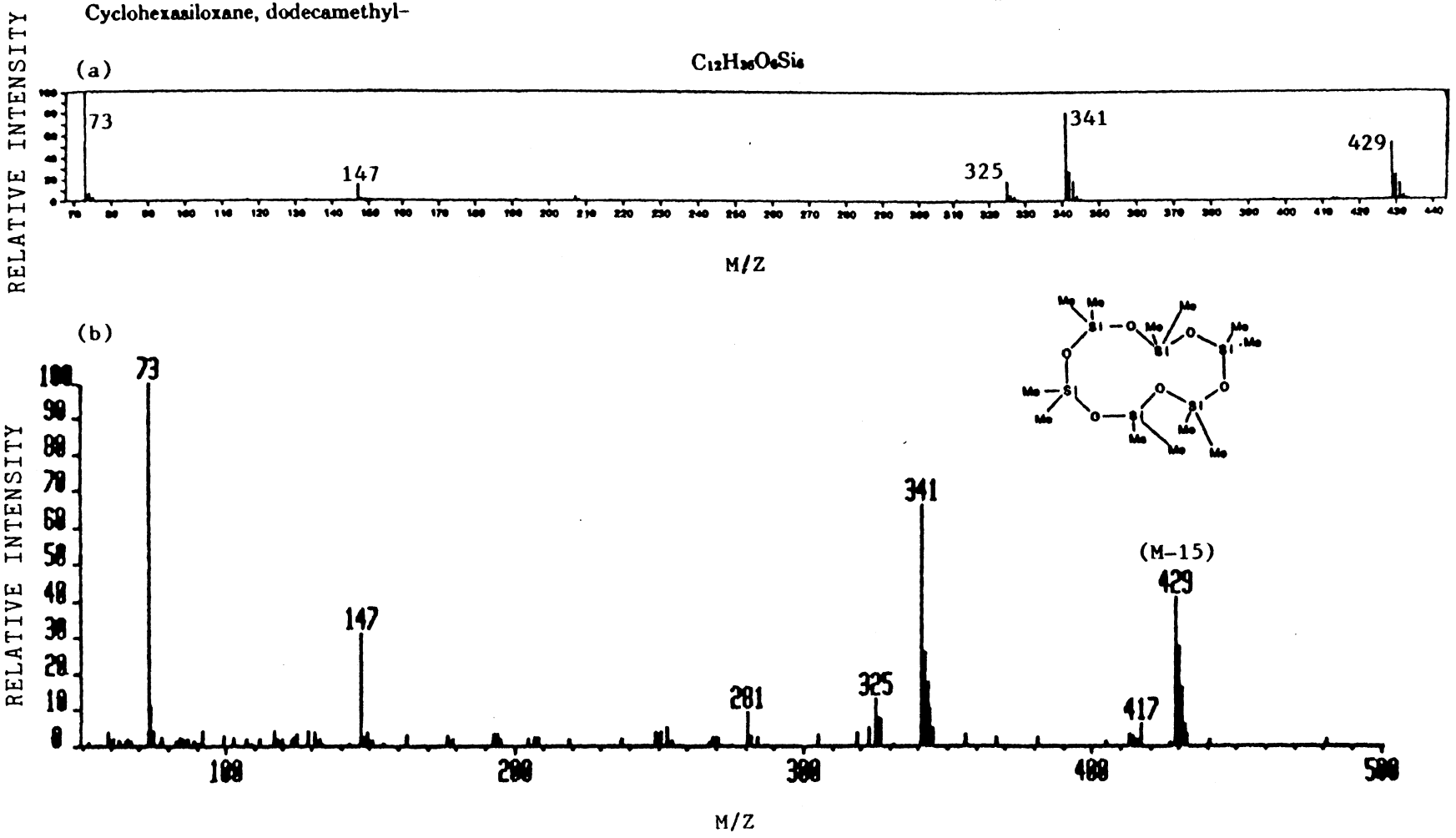


TABLE IX

SUMMARY OF CHEMICAL IDENTIFICATIONS
BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Compound Name	M.W. Determined	Fraction of Extract				Discussion and Spectrum Shown on Page
		New Straw	Old Straw	CT Soil	NT Soil	
Malonic acid	132*	methanol	methanol	---	---	54-67
Fumaric acid	144*	methanol	---	---	---	54-67
Succinic acid	146*	methanol	methanol	---	---	54-67
Malic acid	162*	methanol	---	---	---	54-67
Nonanedioic acid	216*	methanol	---	---	---	67-69
β-D-Talofuranose, 1,2:5,6-bis-o-(1-methylethylidene)	260**	methanol	methanol	---	---	67, 70-71, 74
β-D-Fructopyranose, 2,3:4,5-bis-o-(1-methylethylidene)	260**	---	methanol	---	---	67, 72-74

TABLE IX (Continued)

Compound Name	M.W. Determined	Fraction of Extract				Discussion and Spectrum Shown on Page
		New Straw	Old Straw	CT Soil	NT Soil	
D-Mannitol, 1,2:3,4:5,6-tris-o-(1-methylethylidene)	302**	methanol	methanol	---	---	74-76
Palmitic acid	270*	methanol, methylene chloride, chloroform	methanol	methanol	methanol	74, 77-78, 105-106
Stearic acid	298*	methanol	methanol	methanol	methanol	74, 79-80, 105-106
11,14-Eicosadienoic acid	322*	methanol	methanol	---	---	74, 81-82, 85
Oleic acid	296*	methanol	---	---	---	74, 83-85
1,4-Benzene-dicarboxylic acid	194*	methanol, methylene chloride, chloroform	methanol, methylene chloride, chloroform	methanol	methanol	85-91, 94, 97-105

TABLE IX (Continued)

Compound Name	M.W. Determined	Fraction of Extract				Discussion and Spectrum Shown on Page
		New Straw	Old Straw	CT Soil	NT Soil	
1,2-Benzene-dicarboxylic acid, bis-(2-methylethyl)	254**	methanol, methylene chloride, chloroform	methanol, methylene chloride, chloroform	methanol	methanol	85-94, 97-105
Unknown	271-273**	methylene chloride, chloroform	---	methanol	methanol	94-96, 104-105
9-Hexadecenoic acid	268*	---	---	methanol	methanol	106-108
Cyclohexasiloxane, dodecamethyl	444**	---	---	methanol	methanol	106, 109-110, 112

All compounds were identified as components of the mixture in which diazomethane was added:

* compounds identified as their methyl esters;

** compounds identified as their original forms, assuming that these compounds were not changed in diazomethane.

CT - conventional-tillage.

NT - no-tillage.

CHAPTER V

SUMMARY

The phenomenon of allelopathy has been implicated as one factor in reduction of yield in no-tillage practices of wheat farming. Wheat straw and soil samples of both no-tillage and conventional-tillage wheat plots were collected at the harvest and extracted in order to study the allelopathic reactions. Slightly basic conditions showed that it was the effective extraction method than slightly acidic conditions.

The results of this study indicated that toxic compounds existed in both wheat straw and wheat soil. Bioassay experiments showed that the new wheat extracts had the strongest inhibitory effects on wheat seedling growth and that the basic extracts of conventional-tillage soil were slightly more inhibitory to the wheat seedling growth than that of no-tillage in the harvest season.

An allelochemical linkage between wheat straw residue and soil was indicated in the characterization of the chemical compounds of the extracts. Several known allelochemicals were found in extracts of new wheat straw and soil samples but not in the old wheat straw extracts. One unknown compound was also found in new wheat straw and in both CT and NT soil, and was very toxic according to the

bioassay results. It can be concluded that toxins may leach from wheat straw residue into the soil through rain or microbial decomposition, resulting in inhibition of the growth of successive crops in no-tillage and conventional-tillage farmings.

The author suggests that more quantitative studies and characterizations on more specific phytotoxic compounds need to be done to determine their chemical nature.

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APPENDIXES

APPENDIX A

DRY WEIGHT (G/M^2) OF STANDARD WHEAT FROM EFAW PLOTS, STILLWATER, OKLAHOMA, FOR 1985-1986

TABLE X

DRY WEIGHT (G/M^2) OF STANDARD WHEAT FROM EFAW PLOTS, STILLWATER, OKLAHOMA, FOR 1985-1986

Date	Dry Weight (g/m^2)	
	Moldboard (conventional-tillage)	No-tillage
January 7	47	40
March 3	380	90
March 19	866	125

Note: Table information courtesy of Dr. Gene Krenzer, Department of Agronomy, Oklahoma State University (Krenzer, 1987).

APPENDIX B

GRAIN YIELD (BU/ACRE) FROM EFAW PLOTS, STILLWATER, OKLAHOMA, FOR THREE CONSECUTIVE YEARS

TABLE XI

GRAIN YIELD (BU/ACRE) FROM EFAW PLOTS, STILLWATER, OKLAHOMA FOR THREE CONSECUTIVE YEARS

Year	Grain Yield (bu/acre)	
	Moldboard (conventional-tillage)	No-tillage
1982-83	36	41
1983-84	55	60
1984-85	35	43
1985-86	18	3*
1986-87	25	16

*Wheat killed by simazine herbicide which was applied on March 3, 1986.

Note: Five-year data courtesy of Dr. Gene Krenzer, Department of Agronomy, Oklahoma State University (Krenzer, 1987)

VITA

Ruowei Lori Zhu Thorne

Candidate for the Degree of

Master of Science

Thesis: ALLELOPATHIC EFFECTS IN ASSOCIATION OF WHEAT SOIL AND STRAW WITH DETERMINATION OF SELECTED ALLELOCHEMICAL STRUCTURES.

Major Field: Biochemistry

Biographical:

Personal Data: Born in Shanghai, China, May 7, 1962, the daughter of Mr. and Mrs. Zuifei Zhu

Education: Graduated from Shanghai Jiao-Tong University Attached High School, Shanghai, China in 1980; received a Bachelor of Science degree in Chemistry from Beijing University, Beijing, China in July, 1984, completed requirements for the Master of Science degree at Oklahoma State University, in December, 1987.

Professional Experience: Graduate Research Assistant, Department of Biochemistry, Oklahoma State University, September, 1985-August, 1987; Laboratory technician, Department of Biochemistry, Oklahoma State University, September, 1987-present.